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Editors

Fetal and Neonatal Pathology

Fourth Edition

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Jean W. Keeling and T. Yee Khong (Eds)

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For Lauren, who continues to delight and inspire
Jean W. Keeling

For Anne, Jonathan, and Jeremy
T. Yee Khong

Preface

It is 20 years since the publication of the first edition of *Fetal and Neonatal Pathology*. Perinatal pathology and its related clinical specialties have seen many changes during that time. Within the practice of pathology, there have been many advances of different levels of importance to our specialty. Currently, the investigation of the molecular basis of normal development and of disease has changed our concepts of both and, at a more practical level, has supplied us with the tools to achieve more precise answers in individual cases. Aspects of perinatal pathology that have benefited particularly from this technology are tumors, skeletal dysplasias, muscular dystrophies, and renal cystic disease, where the effects have extended far beyond the purely diagnostic. Molecular pathology has resulted in new insights into developmental anomalies, as well as new tests for families at risk.

The overall format of the fourth edition remains unchanged from the third edition, with chapters in the first half of the book addressing more general problem areas in perinatal pathology, followed by system-based chapters covering specific pathological abnormalities.

In this fourth edition, editorial responsibility has been shared by Drs. Keeling and Khong, as they have interests that are complementary. A new editor brings new ideas and contacts. There have been changes in authorship of a number of chapters, reflecting the changing interests of individuals as well as the passage of time. It is particularly pleasing to welcome a number of young authors, who bring different approaches and new ideas.

A new chapter addresses the clinicians' view of the perinatal autopsy and a new section covers perinatal hematological problems. The continued importance of the adverse effects of infection in the perinatal period is reflected by an expansion of this section, as well as a separate chapter on placental infection and its consequences.

Color illustrations were introduced in the third edition but, in this current edition, there has been a major move to color, resulting in improved clarity in many cases.

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The Clinicians' View of Fetal and Neonatal Necropsy

E. Sarah Cooper and Ian A. Laing

In many parts of the developed world the role of the perinatal necropsy has diminished in status, for a number of reasons. There may be a touching faith in the accuracy of noninvasive technology to provide all answers. There may be a suspicion that clinicians wish to experiment on deceased fetuses and newborns. These understandable beliefs may in part have stemmed both from the failure of obstetricians and neonatologists to explain to families the limitations of sophisticated equipment and from secrecy around the process of necropsy. Such secrecy was often well intentioned, and generally stemmed from a misguided wish to protect vulnerable parents from the details of the examination of their baby's body. In 2003 the *British Medical Journal* devoted an issue to the subject of death. It included five editorials and five original articles on mortality: the subject of necropsy was not mentioned once (Anon 2003).

There is an easy path for all clinicians. We can avoid asking for autopsies because the "baby has suffered enough" and suggest that our clinical skills, imaging, phlebotomy, microbiology, and histological capabilities have already given us the complete answer to the causes of the demise of the fetus or neonate. To take this line is to abrogate our responsibilities to the family. It is essential that we as clinicians should persuade our professional colleagues of the high value of necropsy, especially to bereaved families. Professionals should emphasize to parents that it is their *opportunity* and *right* to have a necropsy carried out on their baby. Only then can the most appropriate counseling be entered into with accuracy, confidence, and in a spirit of trust.

Increasingly, obstetricians and neonatologists work together as a perinatal team, along with pathologists, radiologists, geneticists, midwives, neonatal nurses, and other disciplines. The ideal perinatal team brings the neonatologist to meet with parents for whom fetal death has occurred or where fetal demise is anticipated. Equally the obstetrician should demonstrate an ongoing commitment to families where a neonatal death has occurred. It may be that the obstetrician's and neonatologist's views, as presented separately below, create an artificial divide where none exists. Nonetheless, it is possible to learn from one another's perspectives and that is the purpose of this chapter.

The Purpose of the Necropsy

The necropsy has contributed to knowledge in several well-documented ways:

- It establishes the definitive cause and manner of death (Landefeld et al. 1988).
- It identifies unsuspected associated findings (Maniscalco and Clarke 1982).
- It provides wider benefits to society, including public health statistics (Anderson 1977; Williams and Perry 1977).
- It elucidates pathogenic mechanisms and new diseases (Lundberg 1983).
- It evaluates the accuracy of clinical diagnoses and the efficacy and safety of new diagnostic or therapeutic interventions (Dahms 1986).

- It contributes to the education of medical students (Lundberg 1983), clinicians (Prutting 1977; Friederici and Sebastian 1984), and pathologists (Berthrong 1984).

The Fetal Necropsy: An Obstetrician's Perspective

Perinatal death is an uncommon event, occurring in around eight per thousand pregnancies (Scottish Perinatal and Infant Mortality Report 2001; Confidential Enquiry into Maternal and Child Health 2005). This low rate, with a trend toward lower family size in most developed countries, has led to the parental expectation that every pregnancy will result in a normal healthy baby (Department of Health 2006). The perception is further compounded by the unrealistic expectation that advances in antenatal screening and diagnostic tests and fetal monitoring techniques will guarantee that nothing goes wrong. The diagnosis of a fetal abnormality or of intrauterine death is, then, devastating for parents, and they expect a full explanation of what and how things have gone wrong.

The value of the perinatal necropsy to the obstetrician extends far beyond the search for a cause of death. It is very valuable in the counseling of parents (Faye-Peterson et al. 1999). It may help in the grieving process and reduce the natural tendency for parents to blame themselves for the baby's death (Beckwith 1989). Genetic or obstetric factors relevant in the management of subsequent pregnancies may also be identified. These can form a basis for planning care. Necropsy may confirm or refute diagnoses made by new techniques and thus contribute to their evaluation as well as forming a basis for research and education. It can also be used to monitor the adverse effects of diagnostic tests and new treatments, and is very important in the audit of clinical practice, particularly when there has been a poor outcome. Conversely the necropsy may provide reassurance to obstetricians about their management of the pregnancy. Finally, it may provide valuable information for epidemiological surveys and national statistics.

The areas of major importance to the obstetrician of the perinatal postmortem are the investigation of stillbirth, the investigation of intrapartum death, the diagnosis of congenital abnormalities, and the audit of obstetric practice and education.

The Classification of Stillbirth

Stillbirths can be classified using three different methods: the pathophysiological method (Wigglesworth 1980), the fetal and neonatal classification (Hey et al. 1986), and the obstetric (Aberdeen) classification (Cole et al. 1986). When classified according to the extended Wigglesworth classification, unexplained antepartum stillbirth is the biggest group (incidence 4.06 per 1000 births); (Confidential Enquiry into Maternal and Child Health 2005) representing 70.7% of all stillbirths in 2003. The largest identifiable causes of death are congenital abnormality (15.2% in 2003) and intrapartum causes (7.6% in 2003). When unexplained antepartum deaths are classified using an obstetric (Aberdeen) classification, the proportion of unexplained deaths falls to 51% of all stillbirths. The remainder include stillbirths due to antepartum hemorrhage, maternal disorder, preeclampsia, and mechanical complications (8%, 5.1%, 3.9%, and 1.1% of all stillbirths in 2003, respectively); (Confidential Enquiry into Maternal and Child Health 2005).

The Investigation of Stillbirth

The causes of antepartum stillbirth are many and varied. However, a large number remain unexplained. Investigations routinely used include maternal serology for viral causes of stillbirth including cytomegalovirus (CMV), toxoplasmosis, rubella, herpes simplex, and parvovirus B19 (Benirschke and Robb 1987). Tests for the presence of antinuclear, antiphospholipid, and anticardiolipin antibodies and lupus anticoagulant are routine in the United Kingdom. The Kleihauer test reveals recent fetomaternal hemorrhage. Maternal blood cultures are done if *Listeria* is suspected. Estimation of maternal blood glucose may reveal previously undiagnosed diabetes. The fetal necropsy, however, is the investigation most likely to provide answers in the investigation of antenatal stillbirth, and pathological examination of the placenta may reveal a significant abnormality

in up to 30% of cases (Incerpi et al. 1998) (see Chapters 3 and 4).

The primary role of the necropsy, and all other investigations, lies in determining a cause of death. Determination of underlying factors that might recur in a subsequent pregnancy is also important. These include fetal growth restriction and placental villitis, a nonspecific finding that may indicate infection or be a sign of maternal preeclampsia. A previously undiagnosed congenital abnormality may also be apparent at autopsy.

In the case of intrapartum stillbirth there may be evidence of an asphyxial or mechanical insult during labor, or that the baby is already compromised and unable to tolerate the hypoxic stress of a normal labor.

Fetal Growth Restriction

Fetal growth restriction is associated with an increased risk of stillbirth and poor perinatal outcome (Clausson et al. 1999). It contributes significantly to the high unexplained stillbirth rate. While late fetal mortality due to other specific causes has declined over the last few decades, unexplained antepartum death rates have remained largely unchanged. Improved identification of deaths due to growth restriction, which may otherwise be classified as unexplained, is important (Bell et al. 2004). When stillbirths are classified using customized weight charts, 43% are found to be growth restricted ($<10^{th}$ centile for gestational age) (Gardosi et al. 2005). This does not explain the precise cause of death, but the knowledge that a stillborn baby showed signs of growth restriction enables the obstetrician to manage subsequent pregnancies in that family with an appropriate level of monitoring. Doppler examination of uterine artery waveform may predict the development of intrauterine growth restriction later in pregnancy (Bower et al. 1993). Serial growth measurements may reveal abnormal growth velocity. Changes in the waveforms of fetal Doppler traces may indicate developing hypoxia. The umbilical artery is a sensitive indicator of placental vascular resistance, and there is an inverse correlation between Doppler measured resistance and fetal venous pO_2 (Weiner 1990). Examination of the fetal middle cerebral artery (Vyas et al. 1990) and venous system (Baschat 2004) enhances the positive predictive value of an elevated umbilical artery Doppler. Biophysical scoring (Manning et al. 1980) may be useful as part of the overall fetal assessment. For the well-grown baby, with no other complications, a normal biophysical score may provide reassurance for a week, but in the compromised fetus it may provide reassurance of well-being for, at most, only 24 hours (Manning 1999).

The majority of parents who experienced a previous stillbirth, regardless of cause, choose to have fetal monitoring and often comply with the most complicated of regimens. Parents seem to find monitoring with ultrasound particularly reassuring.

Placental Pathology

The examination of the placenta can provide useful information in up to 30% of cases (Incerpi et al. 1998). In this study, abnormal placental findings included chorioamnionitis, multiple infarcts, hemorrhage, clots, acute or chronic villitis, and occlusive vasculopathy. The finding of chorioamnionitis is indicative of infection; retroplacental hemorrhage and clots suggest placental abruption and infarcts, and vasculopathy and villitis are seen in the placentas of fetuses affected by growth restriction and preeclampsia. The great value of placental examination should be emphasized to parents when they feel unable to give consent for fetal postmortem examination (see Chapters 3 and 4).

Intrapartum Stillbirth

Death from intrapartum causes accounts for 7.6% of all stillbirths and 9.1% of all neonatal deaths (Confidential Enquiry into Maternal and Child Health 2005). Intrapartum related death is defined as "any baby who would have survived but for some catastrophe occurring during labour" (Confidential Enquiry into Maternal and Child Health 2005). This is usually severe hypoxic stress, and is more likely when an already compromised fetus is unable to withstand the normal hypoxic stress of labor. Other causes are mechanical complications such as cord prolapse and shoulder dystocia. A minority of intrapartum deaths follow intrapartum trauma, especially during vaginal breech or

forceps delivery. With good obstetric care in labor, these deaths are currently rare. There has been a dramatic reduction in intrapartum-related mortality over the past decades, from a rate of 10.2 per 1000 births in the 1958 Perinatal Mortality Survey (Butler and Bonham 1963), to 0.6 per 1000 births in 2003 (Confidential Enquiry into Maternal and Child Health 2005). This reduction has occurred not only because of improvements in intrapartum management, but also because of the improved health of the population. Unfortunately, whereas the general public believe that loss of a baby because of congenital abnormality or extreme prematurity is unavoidable, loss of a baby because of an intrapartum event is often assumed to be the result of substandard care in labour (see Chapter 13). Such deaths often create feelings of anger in the parents and cause great distress to the staff involved. They may also form the basis for lengthy and expensive litigation. Postmortem examination of these infants can provide very important information about the time scale of the pathophysiological process leading to death, which may have very significant implications for the staff involved.

Cord Knots and Cord Entanglement

The presence of a true knot in the cord, or the finding that the cord was around the baby's neck at delivery, is frequently discussed at perinatal mortality meetings, and the significance of such findings is enthusiastically debated. Nuchal cord entanglement is commonly seen at delivery. A single encirclement is seen in 23.6% of deliveries and multiple encirclement is seen in 3.7% (Carey and Rayburn 2000). The significance of nuchal cord is controversial, with reports of an increased incidence of fetal heart rate abnormalities in labor (Larson et al. 1995), but no significant risk of stillbirth or neonatal morbidity (Larson et al. 1997, Carey and Rayburn 2000). There is little evidence to support the use of routine ultrasonographic nuchal cord assessment (Schaffer et al. 2005).

The incidence of true cord knots is 1.25%, and the majority of these will either present incidentally or will cause temporary fetal distress during labor with no significant problems after birth. They are associated with multiparity, postdates pregnancy, male fetus, and long cord (Airas and Heinonen 2002). They are only significant if tight enough to obstruct the fetal circulation. Fetuses with a true cord knot have a fourfold increased risk of stillbirth (Airas and Heinonen 2002). Unfortunately, true knots do not have a characteristic appearance ultrasonographically and are easily missed on routine screening (Sepulveda et al. 1995). In cases of both cord entanglement and true knots, the pathological finding of edema and congestion on one side of the obstruction and thrombosis of vessels in the vicinity increases the likelihood that it may have been responsible for fetal death.

Intrapartum Hypoxia

Intrapartum hypoxia does not always result in death. Significantly for the parents of a surviving asphyxiated baby, their child may be not be neurodevelopmentally normal. Opinions as to the timing of brain injury differ, with some believing that events in the immediate perinatal period are the most important in neonatal brain injury (Cowan et al. 2003). The Scottish perinatal neuropathology study looked at the brains of asphyxiated and nonasphyxiated infants and concluded that in a large proportion of neonatal deaths, brain injury predates the onset of labor and was more common in babies born in an asphyxiated condition (Becher et al. 2004a). This supports opinion that not every case of neonatal encephalopathy is caused by birth asphyxia and that most cases of cerebral palsy relate to antenatal events (Hall 1989; Nelson and Leviton 1991; Yudkin et al. 1995; Edwards and Nelson 1998; Bakketeig 1999; Maclennan 1999). Although we should continue to improve intrapartum care to reduce the number of infants that are damaged by intrapartum events, we must develop methods for the antenatal detection of these infants to optimize their care.

Congenital Malformations

Congenital malformations account for the largest of the identifiable causes of fetal death (15.2%; Confidential Enquiry into Maternal and Child Health 2005). The number of potential genetic diagnoses is vast, with recurrence risks varying between sporadic and 100%, depending on the inheritance pattern of the disorder. Only when an accurate diagnosis has been made can the parents be appropriately counseled as to the cause of

death and likelihood of recurrence. The purpose of necropsy is to diagnose unsuspected anomaly and confirm any previously diagnosed abnormality, noting any coexisting malformations or dysmorphic features that may alter the recurrence risk (Shen-Schwartz et al. 1989). In a study of 172 sequential cases, which included 52 fetal and 87 neonatal deaths, 81% were examined postmortem. In 26% the necropsy was the sole means of establishing the cause of death. In almost half of the 62 cases that required specific genetic counseling because of structural abnormalities, the necropsy was the sole source of information highlighting the importance of this procedure (Meier et al. 1986).

Infection

Approximately 2% of stillbirths are caused by infection (Confidential Enquiry into Maternal and Child Health 2005), and a large number of different pathogens have been implicated (Goldenberg and Thompson 2003). However, the relationship between stillbirth and infection is not always clear. Necropsy may reveal evidence of infection in addition to other pathologies, for example hypoxia, leaving uncertainty about the relative importance of each to the cause of death. Isolating organisms from the fetus or placenta or finding histological evidence of chorioamnionitis does not prove causation. Nevertheless, several studies have found that the incidence of chorioamnionitis after stillbirth is higher than in controls, and that this association is much stronger if there is inflammation of the chorionic plate or fetal organs (Moyo et al. 1996; Maleckiene et al. 2000; Tolockiene et al. 2001). Histological chorioamnionitis is very common with an incidence of 5% of term pregnancies and more than 50% of preterm deliveries. There may be no sign of clinical infection and it may occur in the presence of intact membranes. Intraamniotic infection (IAI) is a clinical diagnosis characterized by maternal pyrexia, leukocytosis, uterine tenderness, and fetal tachycardia, and occurs in 1% to 2% of deliveries (Gibb and Duff 1991). The difficulty in interpreting the results of studies of infection in pregnancy arises because many do not distinguish between histological chorioamnionitis and IAI. Thus, the significance of chorioamnionitis at necropsy is also not always clear, particularly in the absence of fetal infection such as pneumonia. The evidence for giving antibiotic prophylaxis in subsequent pregnancies is often weak. There are exceptions, and when there is a clear link between infection and stillbirth, strategies exist for reducing risk in subsequent pregnancies, for example group B streptococcal infection (Royal College of Obstetricians and Gynaecologists [RCOG] Green Top Guideline 2003).

There is a clear association between some viral infections, for example parvovirus B19, rubella, chickenpox, enteroviruses, and stillbirth. Usually previous infection elicits an antibody response that is protective in a future pregnancy. An exception to this is CMV; congenital CMV may occur with recurrent infection. However, although CMV is the commonest cause of intrauterine infection, the link between CMV and stillbirth has not been firmly established (Nelson and Demmler 1997). Infections in pregnancy and the neonatal period are discussed in detail in Chapter 16.

Management of Subsequent Pregnancies

Using the information gained from the necropsy, maternal serology, and bacteriology, an appropriate plan for management of subsequent pregnancies can be made. This may include prenatal diagnosis by amniocentesis or chorionic villus sampling for karyotyping or DNA analysis in the case of a previous aneuploidy or single gene defect. Detailed anomaly scanning may provide reassurance if there has previously been an ultrasonically detectable structural anomaly. Serial growth measurements showing a normal growth velocity, and normal umbilical artery Doppler resistance index may help to exclude fetal growth restriction. In women with antiphospholipid antibodies, first and second trimester miscarriage and late stillbirth are more common. In placentas from these patients, a decrease in vasculosyncytial membranes, fibrosis, hypovascular villi, an increase in vasculosyncytial knots, and thrombosis and infarction are more commonly seen than in patients without antiphospholipid antibodies. It is suggested that these findings are likely to result from prolonged hypoxia due to thrombosis or infarction, and antithrombotic treatment is therefore a logical approach (Out et al. 1991). In subsequent pregnancies treatment with low-dose aspirin (from preconception) and low molecular

weight heparin (when pregnancy is confirmed) is a reasonable approach, after first counseling the patient on the risk of heparin-induced osteoporosis (Nelson-Piercy 2002). Whether this extends to other thrombophilic conditions associated with adverse pregnancy outcome, such as the inherited thrombophilic defect factor V Leiden, is not clear, and further investigation is required (Khare et al. 2003). It has been suggested that aspirin and heparin may be useful in any woman with a history of placental infarction and thrombosis in a previous pregnancy, but there has been no randomized controlled trial of this treatment, and long-term heparin use has its risks. Studies of the use of low-dose aspirin alone for women with a history of preeclampsia or a previous severely growth retarded baby have shown conflicting results of efficacy (Imperiale and Petrulis 1991; Italian Study of Aspirin in Pregnancy 1993; Leitich et al. 1997; Duley et al. 2001), but it appears to be safe for use in pregnancy (Collaborative Low Dose Aspirin Study in Pregnancy Collaborative Group 1995).

There is always a high level of anxiety in subsequent pregnancies, and parents often find frequent monitoring, particularly with ultrasound, very reassuring. However, none of the available antenatal monitoring techniques can guarantee that nothing will go wrong. No amount of reassurance from monitoring is a substitute for seeing the baby safely delivered. A significant number of parents, particularly those whose previous loss was unexplained, feel very anxious if the pregnancy is prolonged past term. In view of the steep rise in stillbirth rate after 37 weeks (Yudkin et al. 1987), and the low risk of perinatal morbidity, most obstetricians would feel that it is reasonable to electively deliver after this time. Some parents choose delivery by cesarean section, particularly those who have lost a baby as a result of intrapartum events.

Prenatal Diagnosis of Congenital Abnormalities

Accuracy of ultrasound in the diagnosis of congenital abnormality is variable (Chitty et al. 1991). After termination of pregnancy for abnormalities detected on ultrasound, additional information that may alter the risk of recurrence is provided by postmortem in 20% to 40% of cases (Manchester et al. 1988; Clayton-Smith et al. 1990; Weston et al. 1993; Medeira et al. 1994; Faye-Peterson et al. 1999; Johns et al. 2004). Prenatal diagnosis and screening are discussed in more detail in Chapter 6.

Termination of Pregnancy for Fetal Abnormality

The decision to terminate a pregnancy for fetal abnormality is often a very difficult one for parents to make. The role of the necropsy is to reassure parents and their obstetrician that the ultrasound diagnosis was correct, and to find any additional abnormalities that may influence the recurrence risk.

First Trimester

The majority of first trimester terminations are for aneuploidy, a karyotypic abnormality being the basis of a decision to terminate the pregnancy. The role of fetal examination is largely to confirm the karyotype. First trimester demonstration of nuchal translucency followed by a detailed scan enables the early identification of structural anomalies in karyotypically normal fetuses. Here, necropsy diagnosis is essential for the confirmation of the diagnosis, the identification of other abnormalities that may affect the risk of recurrence, and audit of the procedure. One of the advantages of early diagnosis for the parents is that it allows the choice of method of termination. Unfortunately, surgical termination by vacuum aspiration often results in fetal disruption, limiting the information that can be obtained by the pathologist. Surgical termination can be modified using ultrasound-guided aspiration to produce a more complete fetus (Soothill and Rodeck 1994). Medical termination usually results in the delivery of an intact fetus, enabling the identification of a range of structural abnormalities as early as 9 weeks' gestation (Blanch et al. 1998). Parents must understand the potential loss of information if they choose a surgical method of termination.

Second Trimester

Medical termination is usually the method used in the second trimester, and the maximum information can be obtained from a fresh specimen. A

few centers offer surgical termination by dilatation and evacuation, resulting in fetal disruption. Confirmation of diagnosis is possible for most disorders diagnosed on ultrasound (Shulman et al. 1990), but minor abnormalities are not always detected, potentially limiting the advice given to parents about future pregnancy.

The Royal College of Obstetricians and Gynaecologists and Royal College of Pathologists (2001) joint working party recommends that nonsurgical methods should be used for termination of pregnancy to allow optimal pathological examination. Surgical methods should be reserved for termination for known aneuploidy or in pregnancies of less than 11 weeks' gestation.

Late Termination

The Royal College of Obstetricians and Gynaecologists (1998) recommends the use of intracardiac potassium chloride after 21 weeks of gestation to ensure that the fetus is born dead. In my experience, the pathologist often comments that this can result in a deterioration of the condition of the fetal tissues, especially the brain and heart. This may in turn limit the ability of the pathologist to provide an accurate diagnosis of particularly cerebral anomalies.

Falling Rate of Necropsy

Over the last few decades, there has been a significant change in attitudes toward perinatal death. A stillborn baby born 50 years ago would have been removed from the delivery room before its mother could see it, and the idea of a funeral service would have been unheard of. It is now normal practice to let parents cuddle and bond with their stillborn baby, often for some hours. At this point to then be asked for authorization for necropsy can be very difficult for parents. They often express the view that the baby has "suffered enough," and think, incorrectly, that the baby will be changed beyond recognition by the procedure. It is important to be sensitive to these issues when discussing necropsy, and to reassure parents that their baby will not be disfigured. Unfortunately there has also been a decline in the understanding of, and a change in the attitudes of staff toward the necropsy (Chiswick 1995; Turner and Raphael 1997). This, together with recent high-profile cases where tissues and organs of babies were retained without the consent of parents, has resulted in a decline in the rate of perinatal autopsies over the past few decades (Stock and Keeling 2006).

Alternatives to Necropsy

The necropsy remains the most effective way of investigating fetal death, with the potential to diagnose a wide range of disorders, but as discussed above the rates of postmortem have declined significantly. Parents who do not give consent for full necropsy usually object to examination of the internal organs. Valuable information, in these circumstances, may still be obtained from external examination, radiology, and examination of the placenta (Wright and Lee 2004). Magnetic resonance imaging (MRI), particularly of the brains and spines of fetuses, can provide high levels of resolution and may be a useful adjunct to ultrasound (Whitby et al. 2004). However, on other organ systems, particularly the heart, results are disappointing. Postmortem MRI has been suggested as an alternative to internal examination for parents who decline this. However, agreement between MRI and postmortem is low, with one study of 20 cases finding agreement in only eight cases (Brookes et al. 1996), and in another 37 of 47 major abnormalities detectable on postmortem were detected by MRI, but only one of 11 minor abnormalities (Woodward et al. 1997). Magnetic resonance imaging may provide some information when invasive necropsy has not been allowed (Griffiths et al. 2005); however, necropsy remains the gold standard (Lyon 2004).

Parental Experience and Opinion

The commonest reason parents agree to necropsy is to obtain answers, as there may be concerns about antenatal or intrapartum events. The second commonest reason is a desire to help others. The commonest reason that parents refuse permission is because of a fear of disfigurement of their baby, the second commonest being that they felt that the diagnosis had already been made (McHaffie 2001). This is sometimes the case after the diagnosis of a lethal abnormality on ultrasound, when the parents may feel that necropsy will not provide any additional information. There is evidence

that, when parents consent to necropsy, they do not regret their decision. In one study of women's reactions to perinatal necropsy, 5% of women who had consented, and 30% of women who had not, subsequently regretted their decision (Abdul and Khong 1995). In another survey, 81% of parents agreed to necropsy, and they viewed the examination as useful and necessary. A significant response was that the necropsy had helped them come to terms with their loss. They felt less guilty when the postmortem confirmed their reasons for termination, or showed that the baby's problems could not be attributed to their actions (Rankin et al. 2002). The use of complicated medical terminology was the commonest reason for parents feeling dissatisfaction with the explanations given to them. The authors suggested a pathologist could be more frequently involved in giving feedback and obtaining consent.

It is clear that obstetricians and midwives involved in the consent process should be fully trained in discussing these sensitive issues, and should be able to explain both the procedure and findings of the postmortem in a clear, uncomplicated manner.

The Role of the Postmortem in Training and Audit

Training of Obstetricians

There is some evidence that obstetricians have a poor perception of the value of the perinatal postmortem in terms of the quality of information produced (Wright et al. 1998). This is inevitably reflected in a low necropsy rate either because the parents are not informed about the benefits of the examination, or because the obstetrician fails to request a postmortem. Doctors who have attended a perinatal necropsy are more likely to feel positive about the benefits than those who have not (Stolman et al. 1994). It is recommended that this should be addressed by the specialist training program, with attendance of at least one perinatal necropsy. There should also be training in obtaining consent for necropsy. In addition, attendance at perinatal mortality meetings and formal teaching by a perinatal pathologist should be encouraged (RCOG and Royal College of Pathologists [RCPath] 2001). It is only with exposure to a well-conducted postmortem examination that a trainee can fully understand the technical skill involved in the examination and be able to obtain fully informed consent from the parents. The trainee will also be more able to allay the fears that parents may have that their baby will be disfigured by the examination. The trainee will also understand the importance of providing the pathologist with all the relevant clinical information, and the pathologist will gain some insight into obstetric practice.

In the U.K., subspeciality trainees in fetomaternal medicine have much greater exposure to perinatal pathology as part of the training program. Many trainees are attached to a perinatal pathology department for a period, and all are expected to attend the necropsies of fetuses terminated for fetal abnormality.

Audit of Clinical Practice

Perinatal mortality meetings are very important in promoting a dialogue among obstetricians, pediatricians, and pathologists. They are the longest established multidisciplinary meeting in the U.K. and elsewhere, and have enjoyed strong support from professional bodies, such as the Royal Colleges. They are most effective when they are nonjudgmental, and they should be seen as an educational opportunity; nursing and midwifery staff should be encouraged to attend. They help clinical staff become familiar with the pathologist's terminology, and the pathologist also gains insight into clinical practice. They are an opportunity to discuss potentially avoidable losses and therefore help to improve future management of all pregnancies, as well as making plans for specific couples. They are thus an important opportunity for the audit of clinical practice. Discussion between clinicians and pathologists at perinatal mortality meetings often result in a more accurate classification of perinatal deaths, improving the reliability of the information collected for these audits.

Neonatal Necropsy: A Neonatologist's Perspective

The following subsections provide further elucidation of the advantages of necropsy that were mentioned above.

Cause of Death

Establishing the definitive cause and manner of death (Landefeld et al. 1988) is clearly of central importance. Parents in the neonatal unit usually form a strong connection with the professional team involved. Generally they trust those who provide high-quality communication and technical expertise during the arduous hours, days, or weeks of the child's life. Nevertheless, the infant's death, whether expected or unexpected, is a moment of challenge as well as grief. The necropsy may provide information to the parents that reinforces this trust, and allows them to continue to think positively about the quality of care and their own future. It is common for parents who have lost one infant to return to the same neonatal unit in future years with another child who also requires excellent care in an atmosphere of strength.

Unexpected Associated Findings

Identifying unsuspected associated findings (Maniscalco and Clarke 1982) may often be of academic interest only. Occasionally, however, they may be of central importance. Parents of an infant with transposition of the great arteries may feel devastated when a balloon septostomy fails and their baby dies soon after. Necropsy may show that the atrial septal defect was, unexpectedly, guarded by a thick fibrous ring, thus explaining why splitting of the atrial septum was unsuccessful. This simple reality, found only at necropsy, may allow parents to accept the inevitability of such a loss.

Wider Benefits to Society

Providing accurate public health statistics (Anderson 1977; Williams and Perry 1977) is of importance to the wider population. For example, a rise in mortality among infants of diabetic mothers might have many explanations, from fetal malformations to neonatal pulmonary disease. Precise quantification of such malformations or diseases has different implications in prevention of such complications. Two separate responses might be to establish meticulous diabetic control around the time of conception, and to assess closely the third trimester fetus with a view to choosing the ideal moment for delivery.

Pathogenic Mechanisms

Elucidating pathogenic mechanisms and new diseases (Lundberg 1983) is part of the pursuit of scientific knowledge. And yet there is a practical significance also. The baby who appears to have severe respiratory distress syndrome in the neonatal period may be thought by the neonatologist to have simple surfactant deficiency. The pathologist may discover during necropsy that the neonate suffered from surfactant apoprotein B deficiency, a diagnosis with profound implications for the family's subsequent pregnancies, given that it is inherited as an autosomal recessive condition. Furthermore, neonatal care in the last 25 years has been enriched by the instillation of animal surfactants, including pig and cow proteins, into the lungs of preterm neonates. Where necropsies have been carried out, it has been reassuring to see little evidence of allergic alveolitis attributable to reaction to such foreign proteins.

Accuracy of Diagnosis

Evaluating the accuracy of clinical diagnoses and the efficacy and safety of new diagnostic or therapeutic interventions (Dahms 1986) should be of great importance to all practicing clinicians. Every cardiologist needs to compare the accuracy of echocardiographic diagnosis against the benchmark of the anatomy of the congenitally abnormal heart. Perinatologists need to ensure that the infant whose death was caused by a diaphragmatic hernia succumbed because of severe hypoplasia of the lungs.

Education

Medical students (Lundberg 1983), clinicians (Prutting 1977; Frederici and Sebastian 1984), and pathologists (Berthrong 1984) need continuing education to develop and maintain their skills, but also to stimulate their enthusiasm for the academic side of their work. It is possible, as we have found, to create an atmosphere in which doctors, nurses, and medical students want to attend the necropsies of the infants they cared for, knowing that they will be taught by an experienced perinatal pathologist about details of the supposed and actual causes of death. The intraventricular hemorrhage, seen hitherto only as an echodensity on

ultrasound examination, becomes a reality during such a tutorial.

Counseling

The neonatal necropsy is recognized to be of great value when counseling families after the loss of an infant (Reynolds 1977; Rowe et al. 1978; Hirsch 1984; Valdes-Dapena 1984). It is very rare for couples, at the bereavement follow-up clinic, to express disappointment that they had authorized a necropsy on their baby. Many express pride that they had made this difficult decision. In our experience, parents appreciate knowing that the neonatologist who cared for their child attended the necropsy. In part, they say, this is because they did not want anything to happen during the necropsy that they had not provided consent for. But, more importantly, parents see the attendance of the neonatologist as an ongoing commitment to the family. They are grateful to receive a telephone call later that day confirming there were no unexpected findings. They are also glad to receive a copy of the pathologist's independent report, especially if it includes a plain-language version of more complex descriptions.

The discussion of findings with relatives often assists the grieving process by helping to alleviate parental concerns and guilt over prenatal events and to clarify and improve understanding about the circumstances around the life and death of their child (Beckwith 1989). Even unexpected findings, if explained sensitively, can be of interest and of some relief to the parents. "They didn't hide anything from us. They even told us that Veronica had an extra tube from her kidneys to her bladder, and nobody knew." "We were pleased that there was nothing we could have done. It wasn't our fault. It'll be OK next time. Yes, we'll be scared. The postmortem showed that we couldn't have changed a thing."

The importance of the postmortem examination also includes the identification of genetic conditions or obstetric factors that may be of relevance to the management of future pregnancies (Saller et al. 1995). The first child to exhibit medium-chain acyl-coenzyme-A deficiency (MCAD) in a family may present in acute collapse and death in the early days of life. The suspected clinical diagnoses may include group B streptococcal sepsis or, in the absence of echocardiography, hypoplastic left heart syndrome. High-quality necropsy may show the histological changes of a primary metabolic condition, triggering the pursuit of the correct biochemical diagnosis. The resultant information is crucial for the family and also for the professionals who will then look after future babies born to this family.

Authorization

Requesting necropsy is fraught with difficulties. Neonatologists do not want to ask because they think that the request will cause distress to the parents. The parents do not want to be asked because "our baby has suffered enough." To the steely pragmatist this is irrational, but the death of a loved member of the family triggers irrational and wholly understandable feelings. The benefits are beyond doubt, as described above, and the neonatologist should recognize that the request, put sensitively and with clarity, may be the most important interview in the lives of the parents, especially if there is great doubt about the underlying cause of death.

Parents are often influenced strongly by the first person to address the subject of necropsy. Much damage can be done by a well-meaning professional who is trying to alleviate the parents' turmoil: "The doctor will ask you about a postmortem, but it's up to you. You can always say no." The doctor who needs information that can only come from the necropsy in order to best counsel parents is now at a disadvantage. How can this be addressed? The answer is clear. The entire team must be committed to the importance of the necropsy. Necropsy requests must never be left to a junior member of medical staff. Authorization of autopsy must be requested principally and firstly by the consultant neonatologist looking after the baby. Only then will the parents recognize the commitment of the professional team to the family over the coming weeks, months, and years. The doors are not closing. The support is ongoing.

The authorization form must be unambiguous. Many different variations of the form are used. Some are so brief that it is unclear that the parents have an appreciation of what they are authorizing. Others are so detailed and proscriptive that

parents comment that they feel intimidated. The authorization form that we currently use is a compromise (Fig. 1.1). An information leaflet is offered at the same time. Copies of both are given to the parents. The responsible clinician must sit with the parents and go over every detail of the process, and allow as much time as is necessary for parents to ask questions and consider their decision.

Authorization should be freely given, and information on every stage of the process should be available and provided on request. The authorization requires that there is no ambiguity in what the next of kin are agreeing to. In particular, the parent or guardian should understand the following points:

- The examination is carried out by a pathologist, a doctor specializing in the study of disease and who is specifically trained to do necropsy examinations.
- After the necropsy is performed, a report will be written by the pathologist and this will be sent to the general practitioner, the pediatrician, and the obstetrician most closely involved. A copy is made available also for the parents. It is good practice for the parents also to receive a version of the report that explains the findings in lay terms.
- The examination should be carried out within 2 working days, and this schedule therefore will be unlikely to affect the timing of funeral arrangements.
- The term *organ retention* refers to whole organs taken from the deceased, and this is done only with appropriate authorization.
- Retention of samples or blocks of tissue or thin slices to make slides is a standard part of a full postmortem examination, and such retention must be authorized by the next of kin. These samples are subsequently regarded as part of the medical record. It should be explicitly stated that these samples will be retained by the hospital and may be used in future years to shed further light on the clinical course, especially if further information or new tests become available with the advancement of medical science.
- Small pieces of residual tissue that are not required for histological examination and are not to be retained will be disposed of lawfully and according to the written protocols of the department, which should reflect national standards.
- It should also be explicitly stated that the hospital or Trust may wish that organs or tissue samples be used for teaching, audit, and research. Authorization for this is obtained from the next of kin and authorization can be withdrawn at any time.

Trends in Neonatal Necropsy Rates

Over the past three decades, both the rate and perceived importance of adult necropsies have declined significantly (Goldman et al. 1983). Financial constraints and the increased sensitivity and sophistication of diagnostic techniques have both led to the cost-effectiveness and clinical value of necropsies being challenged (Burrows 1975; Craft and Brazy 1986; Meier et al. 1986; Dhar et al. 1998). Conversely, the neonatal necropsy rate has generally remained far higher, and studies from North America and Australia have found rates ranging from 59% to 81% (Maniscalco and Clarke 1982; Craft and Brazy 1986; Meier et al. 1986; Van Marter et al. 1987; Khong et al. 1995; Saller et al. 1995; Dhar et al. 1998). In one of the few publications from a British center, Porter and Keeling (1987) calculated the rate to be 90% in Oxford during the early 1980s. The fact that the individual characteristics of different institutions and populations vary should be noted when comparing rates (Battle et al. 1987; Kumar et al. 2000).

Kumar et al. (2000) reported that the necropsy rate at a neonatal unit (NNU) in Illinois had declined significantly between 1984 and 1993 despite the fact that in 44% of cases new information was obtained at such a necropsy. In Scotland, the necropsy rate for stillbirths and neonatal deaths in 2001 was noted to be 50.8% "continuing the decline year on year from the rate of 72.4% in 1997" (Scottish Programme for Clinical Effectiveness in Reproductive Health 2002). Brodlie et al. (2002) subsequently reviewed the case records of 314 neonatal deaths in Edinburgh between 1990 and 1999. A necropsy was performed in 67% of cases, but the rate declined throughout the decade by an average of 2.8% per year.

It is still not fully known what determines whether parents choose to have a necropsy carried out on their baby's remains. In Brodlie

This form must be accompanied by an information leaflet to be kept by the baby's parent(s). A copy of the completed form must be given to the parent(s). A copy must be kept with the notes of any post mortem examination undertaken and with the baby's medical records.

Personal details of baby

Name (please print) ……..
Date of birth …… Date of death……
Hospital record number ……
CHI number (where available) ……..

Information

I/we have discussed with Dr ……..the details of what a post mortem examination involves.
I/we have been given a copy of *Information Leaflet for Parents about Post Mortem Examination,* and have had its contents explained to me/us in full.

FULL POST MORTEM EXAMINATION

I/we understand that a full post mortem examination includes the removal of organs from the body, these organs are then examined, and then they are normally returned to the body at the end of the examination. I/we understand that samples of fluid or small pieces of tissue may be retained for processing so that examination under the microscope can be carried out.

I/we agree / do not agree to a full post mortem examination being carried out on this basis.

I/we understand that the Pathologist carrying out the post mortem examination may wish to retain an organ or organs for further diagnostic examination.
I/we authorise :

- No organs to be retained …… ☐
- Specific organs may be retained: heart ……... ☐
  brain …… ☐
  other (specify) …… ☐
- Any organs may be retained …….. ☐

I/we understand that tissue samples in the form of blocks and slides will be retained from these organs and kept as a part of my/our baby's clinical record.
These organs and/or samples may be used :

- for purposes of research Agree ☐ Do not agree ☐
- for purposes of medical education and training. Agree ☐ Do not agree ☐

Please tick the appropriate box(s):

a. I/we authorise the hospital to arrange for respectful disposal of any retained organ(s). Yes ☐ No ☐

b. I/we wish the organ to be returned to us for burial or cremation. Yes ☐ No ☐

c. I/we wish to delay the main funeral service until the organ(s) can be reunited with the body. Yes ☐ No ☐

Parent's name (printed)……..…… Parent's name (printed) ……..…..……

Parent's signature…… Parent's signature ……..…

Witness's name (printed)……. Witness's signature……...……..…..

Witness's designation……...……

A

FIGURE 1.1. (A) Authorization for a full postmortem examination after a stillbirth or neonatal death. (B) Authorization for a partial or limited postmortem examination after a stillbirth or neonatal death. Community Health Index (CHI).

PARTIAL POST MORTEM EXAMINATION

I/we agree to a partial post mortem examination, which includes only the following area or areas of the body:

head	Yes	☐	No	☐	chest	Yes	☐	No ☐
abdomen	Yes	☐	No	☐	other (specify)……..	Yes	☐	No ☐

I/we understand that organs will be removed from the body for examination.
Please tick the appropriate box:

- I/we authorise the Pathologist to retain this organ/these organs for further examination:
  (specify) …… Yes ☐ No ☐
- I/we authorise the Pathologist to retain small pieces of tissue from this organ/these organs:
  (specify) ……..……. Yes ☐ No ☐

These organs and/or samples may be used :

- For purposes of research. Yes ☐ No ☐
- For purposes of medical education and training. Yes ☐ No ☐

I/we authorise inspection of these organs, but do not wish any tissues to be retained for any purpose : Yes ☐ No ☐

Please tick the appropriate box(s):

a. I/we authorise the hospital to arrange for respectful disposal of any retained organ(s). Yes ☐ No ☐

b. I/we wish the organ to be returned to us for burial or cremation. Yes ☐ No ☐

c. I/we wish to delay the main funeral service until the organ(s) can be reunited with the body. Yes ☐ No ☐

Parent's name (printed)……..…….. Parent's name (printed)……..……..

Parent's signature……..……...…… Parent's signature ……..……..

Witness's name (printed)……..… Witness's signature……..…..……..

Witness's designation……

LIMITED POST MORTEM EXAMINATION

- I/we agree to external examination only Yes ☐ No ☐
- I/we agree to photography Yes ☐ No ☐
- I/we agree to X-ray examination Yes ☐ No ☐
- I/we agree to other radiological imaging (specify)…….. Yes ☐ No ☐
- I/we agree to samples of tissue being obtained through a small incision (approximately 1 inch or 2-3cm in size).
  Muscle ……. Yes ☐ No ☐
  Skin ……. Yes ☐ No ☐
  Other tissue sample (specify) …….. Yes ☐ No ☐

Parent's name (printed)……...… Parent's name (printed)……..

Parent's signature……..……...… Parent's signature ……..……..

Witness's name (printed)……..… Witness's signature……..……..…..

Witness's designation……..……

B

FIGURE 1.1. *Continued*

et al.'s (2002) study the only patient-, maternal-, or physician-related factor that was found to be statistically significantly different between the consenting and nonconsenting groups was mean gestational age at the time of birth, 32 and 30 weeks, respectively. This result agrees with the findings of Van Marter et al. (1987) and, for transported infants only, Maniscalco and Clarke (1982). It has been postulated that clinicians are less likely to push for necropsy consent in extremely preterm infants (Van Marter et al. 1987). In the Edinburgh study, the incidence of previous fetal loss was not found to differ significantly between the groups. This is at variance with the findings of Van Marter et al. (1987). It has been argued that lack of parental contact with medical staff may have an adverse effect on necropsy consent (Maniscalco and Clarke 1982), but Brodlie et al.'s (2002) findings suggest that both length of stay in the NNU and transportation were not significant factors.

Demographic features such as the sex of the infant, maternal age, and marital or employment status have never been identified as significant determinants of necropsy authorization (Maniscalco and Clarke 1982; Van Marter et al. 1987; Khong et al. 1995). In Brodlie et al.'s (2002) study, all seven of the Muslim families refused necropsy permission. Religious proscriptions concerning necropsies have been implicated in the general decline of necropsy rates. Geller (1984) argues that with the exception of Orthodox Judaism and Islam, most religions do not prohibit autopsies per se and that in some cases resistance is based on misinformation. Geller is incorrect in implying that autopsies are forbidden in Islam (Ghanem 1988; Rispler-Chaim 1993). There is no reference to this in the Koran and discussion with local Muslim elders has in some cases allowed necropsy to proceed. Islamic law is partly derived from *ijtihad,* the process of deductive logic. *Ijtihad* opinion emerges from the writings of a scholar or a consensus of scholars, although the resulting ruling, called a fatwa, is not binding. In 1982 a fatwa committee declared that the benefits of autopsy might outweigh the drawbacks (Al-Adnani and Scheimberg 2006). Since neither the Koran nor the Sunnah specifically addresses the issue of necropsy, it may be that such an examination would be permitted after close communication with Muslim parents and their religious advisors.

Parental consent is thought to be the major limiting factor of neonatal necropsy rates (Khong et al. 1995). The lay public's exposure to the purposes and value of the necropsy has been sparse, and perceptions are often dominated by melodramatic treatment in the media (Brown 1984). The strength and intensity of individual necropsy requests is likely to be variable, depending largely on the personal bias of the requester as to the importance of necropsy in a specific case. Van Marter et al. (1987) examined the attitude of physicians toward neonatal necropsies by means of a simple questionnaire: senior staff were found to attribute the highest importance to the necropsy. There is no available evidence, however, that those parents counseled by senior clinicians are more likely to grant permission for neonatal necropsy. In Brodlie et al.'s (2002) study there was no significant difference in the rate of consent between families counseled by consultant physicians and those counseled by junior doctors. Today all necropsy consent is sought by consulting physicians, and our group would be reluctant to carry out the study to answer this question.

There may now be some evidence of a reversal in the downward trend of necropsy authorization in the U.K. In 2004 our group reported an audit of 100 consecutive neonatal deaths from January 2000 to June 2004. During this time the perinatal pathology service relocated from the Royal Hospital for Sick Children to the Royal Infirmary of Edinburgh, which contains the Simpson Neonatal Unit. There was a rise in full necropsy rate from 59% pre-relocation to 79% post-relocation (Becher et al. 2004b). We attributed this rise to a new commitment by all doctors, neonatal nurses, and midwives to attend the necropsy whenever possible. This has raised the awareness of staff, and hence the enthusiasm for the process of necropsy, an enthusiasm that can then be conveyed to the bereaved parents.

Do parents in retrospect regret consenting or refusing authorization for neonatal necropsy? In 2002 Rankin and coworkers reported the results of a mail questionnaire given to 258 women who had attended a bereavement counseling service in the north of England. Nine of the 120 respondents who had agreed to a necropsy regretted their decision, while four of the 28 who had refused authorization regretted this decision. Although this has

not been systematically studied, in Edinburgh in the last 20 years no parent who has authorized a neonatal necropsy has expressed such regrets. In McHaffie's (2001) study, conducted by in-depth face-to-face interviews 13 months after the death of the baby, no parents of babies who had undergone necropsy (50 mothers and 40 fathers) expressed regret that they had consented to the procedure.

The Necropsy's Impact on Funeral Arrangements

In general the necropsy should not delay any funeral arrangements. There is, however, one major caveat that must be made clear to the parents. The neonatal brain is soft in consistency and cannot be examined optimally without being "fixed" for a few weeks. A shorter period of fixation may allow less useful examination. Parents therefore have a number of choices open to them. They may opt to authorize a postmortem examination of the brain without fixation or with fixation for a few days only. They may choose to authorize a postmortem that excludes any examination of the brain. The body can then be released for the funeral, with all organs present. Alternatively the parents may opt to allow the brain to be retained by the pathologist so that it can be preserved and examined fully. Under these circumstances the parents need to choose whether they wish the body to be retained for a few weeks so that the brain can be restored to the cranial cavity prior to the funeral. They may prefer to have an early funeral for the baby's remains and a subsequent ceremony for the brain, or they may authorize the hospital to dispose of the brain after the histological examinations are complete. All of these possibilities must be explored in detail with the family, and it is the attending consultant's responsibility to ensure that the family's wishes are carried out precisely.

Communication

The pediatrician has the responsibility to ensure that the pathologist is provided with a detailed account of the clinical information available, including questions (both from parents and from professionals) to be addressed by the necropsy. Attendance by the pediatrician at the necropsy can be a great advantage and is always to be encouraged, especially since professional dialogue during the examination may enrich the findings.

Benefits of the Neonatal Necropsy

Earlier in this chapter the purpose of necropsy was described in six bullet points. But is there an evidence base for these points? Do we know how often new information is discovered at a neonatal necropsy, or how much of this new information is valuable to the family? Does it help them in planning future pregnancies? Is any new information a comfort to the family? A number of studies have begun to address these issues.

In a retrospective study over a period of 10 years, Brodlie et al. (2002) recorded that new information was found in 26% of 314 neonatal necropsies. It was acknowledged, however, that only in one case was the diagnosis incorrect, although in a further five necropsies there was revealed new information crucial for future genetic counseling. If this study proved to be representative, one might argue that in only 2% of necropsies is information discovered that changes the future management of a family.

But it must also be recognized that parents may wish other benefits to emerge from their child's necropsy. McHaffie (2001), in a study involving bereaved parents in the east of Scotland, documented that the reasons why parents consented to necropsy were not confined to obtaining answers and determining genetic factors. Parents volunteered also that they wanted to help other families, to give meaning to the baby's existence, and to confirm that the right decision was made. These factors are hard to measure.

Training

In Edinburgh, all professionals including medical students and nurses in training are encouraged to attend necropsies so that they can appreciate their importance. It may be that as the past adverse publicity recedes and lessons are seen to have

been learned, parents will recognize the commitment of the professionals to provide them with answers. It is to be hoped that full autopsy examination will in the future be authorized in most perinatal deaths, thus improving the quality of care for the bereaved families. End-of-life issues should become part of the undergraduate curriculum for all medical students. This should be accepted by the deans of the medical schools and Trust medical directors. Role play using actors and medical professionals can be used as an important training tool. Junior doctors should also be encouraged to be present at all aspects of communication with grieving parents, especially when they too have been involved in the child's care and they too are experiencing feelings of inadequacy, failure, and grief.

Conclusion

For six centuries the necropsy has been important in the advancement of medical knowledge. Families and society have benefited from the derived information. The new spirit of openness will be reassuring to parents, and we must continue to show an ongoing commitment to necropsies. Most of us learn at least in part by example. It is the responsibility of the attending neonatologist to show unwavering commitment to the care of the dying, communication with the next of kin, and pursuit of high quality in all aspects of the necropsy. Formal and informal training of junior medical staff will ensure that this commitment continues over the coming decades.

Management of the baby's clinical course should always be of the highest standard, and communication between staff and parents must have been exemplary. Only then can the atmosphere of trust be created whereby a family can be guided toward making an informed decision about whether or not their baby should have a necropsy. The parents will remember professional sensitivity or insensitivity for the rest of their lives.

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2
The Perinatal Necropsy

Jean W. Keeling

The importance to clinical practice of information obtained by adequate necropsy examination of babies dying in the perinatal and neonatal periods (Alberman 1980) is often not fully appreciated by histopathologists providing a biopsy-oriented service for adult patients. Examination of stillborn infants has been particularly neglected. This is probably related to negative expectations concerning the likelihood of making specific diagnoses.

Necropsy information is of practical importance at three different levels of clinical practice. First, it is important to the family of the dead baby and to the clinicians involved in their care. Second, necropsy findings are necessary for adequate audit of unit policies and practices. Third, data derived from postmortem examination make an important contribution to regional or national statistics by complementing clinical data collection. Some necropsy data are appropriate to all three levels of enquiry, but each requires answers to specific questions.

In developed countries, where perinatal mortality rates are low, the anticipated outcome of pregnancy is a normally formed healthy infant. The possibility of perinatal death is not seriously entertained unless there has been such a death in the immediate family. Parents want to know what and how things went wrong, without necessarily wanting to attach blame to a particular individual or institution. Of even greater concern is their need to know the risks of repetition of perinatal death in subsequent pregnancies, and, particularly when death occurs in a first pregnancy, their chances of achieving their desired family size.

From the point of view of providing optimal management in subsequent pregnancies, the obstetrician needs to know whether the clinical estimate of gestation and fetal growth were correct, and if the results of prenatal investigations accurately predicted fetal growth and maturity and the presence or absence of malformation (see Chapter 1). The obstetrician also needs to know whether uterine response to gestation was appropriate and whether there was evidence of infection in either the fetus or the gestation sac. In some circumstances the obstetrician is particularly concerned about the possibility of asphyxial or mechanical insult during labour. The neonatologist needs confirmation of diagnoses made during life and whether any treatable conditions went unrecognized. The neonatologist is also concerned about complications of treatment and will welcome information about gestation-related characteristics (see Chapter 1).

National perinatal mortality data are an important index of the nation's health. Accurate collection of information, both in respect to completeness of ascertainment and to the causes of death, is a necessary part of this process. Accurate completion of death certificates and their subsequent registration are essential, and it is important that the causes of death are verified by postmortem examination. It is in both local and national interest to improve the accuracy of perinatal mortality data.

In many countries perinatal mortality rates are currently below 10 per 1000 births. In England, Wales, and Northern Ireland, the perinatal mortality rate in 2002 was 7.87/1000 births (Confidential Enquiry into Maternal and Child Health 2005). This means that the number of deaths in individual units and even regional totals are so low that detailed analyses are readily distorted by events that, in the long term, are of little relevance to

practice. Because of these low levels of perinatal mortality, we are sometimes urged to measure morbidity rates instead (Chamberlain 1985). While it is important to try to collect this sort of information, it should be an adjunct to the collection of mortality data and not an alternative method of measuring clinical practice. Some of the problems related to the accuracy of perinatal mortality data are discussed in Chapter 9. These problems are small when set beside the difficulties in obtaining accurate information concerning morbidity.

An aspect of necropsy examination that is often forgotten is its importance to the continuing education of doctors and other hospital staff, irrespective of seniority (see Chapter 1). To this end, it is important that perinatal necropsies are not just delegated to junior staff and then forgotten. Performance of necropsies should be part of the training of all junior histopathologists, with careful supervision and demonstration of appropriate techniques by an experienced perinatal pathologist.

When clinicians take time to attend postmortem examination on their patients, it does a great deal to encourage the pathologists, to inform them about current clinical practice and about the clinical relevance of information that they can readily obtain. In this way, both the clinicians and the pathologists gain satisfaction, and the value of the necropsy examination is enhanced. Attendance of senior clinical staff in the mortuary demonstrates to junior doctors the importance of postmortem examination in a way that no amount of exhortation from pathologists can ever do.

Importance of Negative Findings

For a pathologist accustomed to the plethora of manifestations of degenerative disease evident at adult necropsy, the lack of findings during perinatal postmortem examination is often a disappointment. It is used as justification for a cavalier approach to perinatal work. Often, the most useful contribution that the pathologist can make is to report negative findings after a carefully executed necropsy.

When a baby dies, the parents contemplate many problems that, in their view, reduce the possibility of successful pregnancy. Clinicians may be worried about the possibility of birth injury or of missed, treatable abnormalities. Careful recording of negative necropsy findings does a great deal to reassure all those involved. To this end, negatives should be clearly and unambiguously stated in the necropsy report, a copy of which is usually filed in the mother's medical chart notes. In subsequent pregnancies the report may be consulted by relatively junior members of both medical and nursing staff to answer specific parental queries or as a basis for management decisions. There must be no room for doubt about negative findings. For this reason, too, the significance of the presence or absence of necropsy findings should be clearly stated, especially those opinions formed after recourse to the literature or discussion with colleagues with particular experience in the field.

Necropsy and Audit

The audit function of necropsy is recognized (see Chapter 1), both in the general hospital (Rushton 1991; Sington and Cottrell 2001) and in specialized institutions (Porter and Keeling 1987; Saller et al. 1995; Tasdelen et al. 1995; Gatzoulis et al. 1996). If perinatal necropsy is to be an effective audit tool, then it is important that a high postmortem rate is achieved. While the rate in fetuses and neonates is higher than in adults, it has clearly dropped during the last 20 years (Khong 1996). The fall is particularly marked among neonatal deaths (Khong et al. 1995; Sutton and Bajuk 1996; Brodlie et al. 2002). Staff education and commitment, based on an understanding of the advantages of postmortem examination, can improve necropsy rates in individual units (Becher et al. 2004).

Although a useful audit tool, it is important that the necropsy is itself audited. Rushton (1991), Vujanic et al. (1995), and Thornton and O'Hara (1998) document the poor standard of perinatal postmortems performed by general pathologists. The common deficiencies are easy to rectify by recording of weights and measurements and by histological examination of fetal organs in antepartum fetal deaths. These "extra" investigations are inexpensive and omitted because of a lack of understanding of their enormous importance, which outstrips the added cost by several orders of magnitude. In regions where most perinatal postmortems are undertaken by specialist patholo-

gists, the difference in standard is striking (Keeling et al. 1994; Vujanic et al. 1998). The increased usefulness of an adequately performed examination is clearly demonstrated by Cartlidge et al. (1995).

Is there a substitute for postmortem examination after perinatal death? When the importance and usefulness of a high-quality perinatal necropsy is so clearly demonstrated, it is important to look critically at claims that imaging is an adequate substitute. Like partial postmortem examination or multiple needle biopsies of organs, imaging does not provide the same detail and standard of information (Furness et al. 1989; Woodward et al. 1997). The suggestion that magnetic resonance imaging (MRI) had equivalent or better diagnostic sensitivity than internal necropsy examination and that the two types of examination were of similar clinical significance (Brookes et al. 1996) is not borne out by Brookes et al.'s own findings. The detection of malformations improves with experience and with the better quality of images as scanners improve, but even major malformations go undetected (Alderliesten et al. 2003). Magnetic resonance imaging can provide detailed evaluation of the central nervous system in situ (Huisman 2004; Whitby et al. 2006), complementing information derived from subsequent pathological and histological examination. Higher-quality resolution and developments such as magnetic resonance (MR) spectroscopy will undoubtedly increase the usefulness of magnetic resonance in fetal examination, but currently necropsy remains the gold standard (Huisman 2004).

Adequate Clinical Information

The importance of the availability of clinical information before the start of any necropsy examination cannot be overstated. This is doubly important to necropsy examination of neonates, when information about the mother is always required and may in some circumstances be of equal or greater importance than that concerning the baby.

The mother's medical chart notes are frequently unavailable before a necropsy examination. Sometimes necropsy examination is undertaken in a hospital far removed from the place of delivery. In these circumstances, clinicians are reluctant to allow removal of the chart from the maternity unit.

Availability of clinical notes does not negate the benefit to both sides of discussion between clinician and pathologist before necropsy commences. Direct communication between colleagues is the best way to ensure that important clinical questions are answered and unrealistic expectations modified before unproductive or even antagonistic attitudes are allowed to interfere. It is important to both clinical and pathological practice that questions are clearly formulated and that techniques are modified to realize the best chance of an unambiguous answer. Clinicians are often disappointed when they do not get answers to questions relevant to patient management following postmortem examination. This disappointment pales into insignificance beside the irritation of pathologists who at the end of their examination are asked a question that they could have answered if it had been put to them at the appropriate time—before they started!

Essential information comprises date and outcome of previous pregnancies as well as details of the index pregnancy. The date of the mother's last menstrual period is the basis for assessment of the length of gestation together with the reasons and methods used for any revision of the original assessment. A history of maternal problems during pregnancy should be sought, particularly hypertension, preeclamptic toxemia, pyrexial illness, diabetes (mellitus or gestational), and vaginal hemorrhage. A note of investigations undertaken during pregnancy and abnormal findings, particularly maternal serum α-fetoprotein levels and ultrasound examinations in the second trimester, are required.

Details of delivery should include date, time, and mode of delivery, reasons for induction of labor or operative delivery, and birth weight. These are all necessary to ensure that the appropriate investigations are undertaken. For live births, details should include condition at birth, appearance, clinical estimation of maturity, and a brief postnatal history addressing problems, procedures undertaken, and clinical opinions about causes of death. Because both positive and negative answers to these questions are important for the conduct of an appropriate necropsy examination, I find that a structured information sheet (Figs. 2.1 and 2.2) is the best way of obtaining essential information at the right time.

REQUEST FOR EXAMINATION OF A FETUS OR PERINATAL NECROPSY

Use Data System if available, and ball point pen

DEPARTMENT OF PAEDIATRIC PATHOLOGY
ROYAL HOSPITAL FOR SICK CHILDREN
2 Rillbank Crescent, Edinburgh EH9 1LF
Tel: 0131 536 0447 Fax: 0131 536 0455
Ansaphone after working hours: 0131 536 0446

Unit Number | LAB Number
First Names | Surname
Address | DoB
Hospital | Ward
Obstetrician | GP | Paediatrician | Baby's Name

PREVIOUS PREGNANCIES

	Date	Gestation	Labour	Puerperium	Sex	Outcome
1.						
2.						
3.						
4.						
5						

<24 weeks
INDICATE IF THIS FETUS IS TO BE RETURNED FOR BURIAL OR CREMATION
YES NO
If NO, or no preference, RHSC, Paediatric Pathology will arrange disposal

REQUEST FOR SANDS PHOTOGRAPH PERMISSION GRANTED
YES NO

PRESENT PREGNANCY Booked/Unbooked, Contraception(Yes/No Type)
INFECTION RISK High/Low Reason
LMP / / EDD / / Gestation: Clinical w. USS w.
Threatened Abortion: Yes/No When? w Amniocentesis: Yes/No Why?
Hypertension: Yes/No US Scan: Yes/No Why?
Pre-eclamptic Toxaemia: Yes/No AFP: Normal/High/Low/Not Done
IUGR: Yes/No Blood Group:
Polyhydramnios: Yes/No Maternal Pyrexia: Yes/No When?
Antepartum Haemorrhage: Yes/No Source When?
Other Problem
Type of Abortion: Spontaneous/Induced for IUD/Social Reasons/Fetal Anomaly (Specify)
Onset of Labour: Spontaneous/Induced.Why?
Last Evidence of Fetal Life: (Date) (Time)
Rupture of Membranes: (Date) (Time)
Liquor: Amount: Normal/Little/Excess Colour
First Stage: (hour) (min) 2nd Stage: (hour) (min)
Fetal Distress: Yes/No Specify
Presentation: Vertex/Breech/Other Delivery
Delivery: Date: Time: Death: Date: Time:

DATE OF REQUEST FOR EXAMINATION: _____ (Neonate: See over)

FIGURE 2.1. Structured information sheet provides minimal clinical data required by the pathologist before perinatal necropsy: pregnancy and labor.

NEONATE: Birth weight g Gestation w APGAR Score at 1 min at 5 min

RESUSCITATION: Nil/Mucus Extraction/0_2 Mask/Intubation

NEONATAL PROBLEMS

1

2

3

4

PROCEDURES

1

2

3

4

SUSPECTED CAUSES OF DEATH

1

2

3

4

ANY OTHER RELEVANT INFORMATION

MEDICAL OFFICER TO BE CONTACTED FOR DISCUSSION OF CASE/VERBAL REPORT

_____ (BLOCK CAPITALS)

FIGURE 2.2. Structured information sheet: neonatal details.

Place of Structured Request Forms in Perinatal Pathology

A carefully completed structured perinatal request form provides the pathologist with consistent, minimal clinical information that is always available before the necropsy begins. Such forms (Figs. 2.1 and 2.2) are not a substitute for access to clinical notes. Their use should not deter clinicians from submitting additional information that they consider relevant.

Besides being a convenient means of transfer of essential information, structured request forms achieve other ends. The process of their completion affords an opportunity to clinical staff in obstetric and neonatal units for a critical review of individual patient management. It may be the first opportunity to look at the case as a whole and observe the way in which problems interrelate at a time when the clinical staff is no longer distracted by a stressful clinical situation. A structured request form also serves to guide the pathologist through the complexities of management of a baby who has received prolonged intensive care, when the clinical notes are likely to be voluminous.

Growth and Development

The fetal and neonatal periods are ones of continuing development at both organ and tissue level, first by a process of cell division and then by growth of individual cells. There is rapid somatic growth, which is roughly linear for most of the second and third trimesters, slowing down from about 38 weeks' gestation until delivery. Knowledge of the length of gestation is an important yardstick against which the appropriateness of developmental markers and somatic growth are measured. Assessment of normality and uniformity of organ development is also important for interpretation of findings at perinatal necropsy. Growth and development advance concurrently and, while they are interrelated, they may be affected together or independently by environmental factors. An awareness of developmental criteria and normal fetal growth is essential to the performance and interpretation of necropsy examination of fetus and neonate. Particular patterns of deviation from the normal might indicate the way in which investigations should proceed or alert the pathologist to commonly associated abnormalities.

In obstetric practice the duration of pregnancy is commonly measured from the start of the mother's last menstrual period. The relationship of this method of measurement to conceptional (gestational) age is dependent on the length of the mother's menstrual cycle and also on her memory. Despite these reservations, menstrual dating usually permits the best estimate of the length of gestation. Ultrasound measurement of the fetus in early pregnancy is commonly performed. It is useful in women with irregular menstrual cycles, but early measurements are more reliable. Current standards for ultrasound dating of pregnancy are as good as menstrual dating (Campbell and Pearce 1985). Alonso and Portman (1995) found biparietal diameter (BPD) a more useful parameter than femoral length. However, these standards are derived from fetal measurements in a population of women of "certain" menstrual dates; a machine-generated number to several decimal places does not imply a dating accuracy down to fractions of a week!

Many of the babies who die in the fetal and neonatal period are of low birth weight. The word *premature* used to be applied indiscriminately to this group of babies, and discrepant historical evidence about the length of gestation was ignored. Prematurity is defined as duration of pregnancy of less than 37 weeks from last menstrual date (Working Party of Obstetricians and Paediatricians in Europe 1970). The Working Party's criterion for a term pregnancy is a duration of 37 to 41 weeks, and it designates pregnancies of 42 weeks and longer as "postterm." Babies who weigh 2500 g or less at birth are designated "low birth weight." Thus, babies of low birth weight may be preterm and appropriately grown, preterm and growth restricted, or mature and growth restricted (small-for-dates).

Birth weight is affected by many different factors, both constitutional (e.g., genetic factors, chromosome abnormality) and environmental. The latter may act directly on the fetus (e.g., first trimester infection affects fetal cell mass), on the placenta (e.g., hematogenous infection compromises fetomaternal transfer of oxygen and

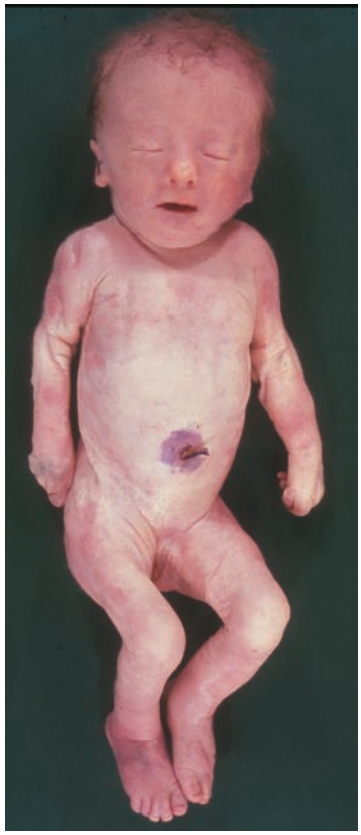


Figure 2.3. Growth-restricted baby, trisomy 18, weighed 1790 g at 39 weeks' gestation (median weight, 3220 g).

nutrients), or via the mother (e.g., chronic undernutrition or poor uteroplacental perfusion from any cause). Small women tend to have small babies, as do women with some inherited abnormalities such as phenylketonuria (PKU). Babies from multiple gestations are of lower birth weight than singletons. Low birth weight is a feature of many malformation syndromes with major somatic defects such as dwarfism and aneuploidy. Fetal growth and factors that adversely affect it are discussed by Kliegman and King (1983). Current birthweight data (Lucas et al. 1986; Yudkin et al. 1987) demonstrate the way in which our concept of the normal weight of preterm infants is altered if standards are based on the weights of all babies in that population. Both groups have shown that babies delivered electively before 34 weeks' gestation are lighter than babies born spontaneously at the same gestation (see Fig. 2.6).

Fetal growth restriction may be of early onset, when it is characteristically "symmetrical," so that fetal organs are smaller but proportionally unchanged. Serial measurements of BPD show that it grows below, but parallel to, the centile lines. This type of growth failure may be the result of intrauterine infection [toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex (TORCH) organisms], genetic constitution, chromosome abnormality (Fig. 2.3) or malformation syndromes such as renal agenesis (Table 2.1). Late-onset growth restriction results in "asymmetric" growth disturbance with relative sparing of brain growth and therefore of head circumference and BPD (Cooke et al. 1977; Fig. 2.4). A disturbance of organ weight ratios is observed, for example an increase in brain to liver weight ratio. Serial BPD measurements are initially within the normal range but progressively fall below normal values in late pregnancy. The causes of this type of growth restriction are usually environmental. It may be the result of reduced uteroplacental perfusion in conditions such as preexisting or pregnancy-associated hypertension, maternal diabetes mellitus, or nonavailabil-

Table 2.1. Features of fetal growth restriction

Characteristics	
Symmetrical	*Asymmetric*
Early onset	Late onset
Constitutional	Environmental
Reduced growth potential	Late growth arrest
Organ weight ratios normal	Brain relatively large
Causes	
Early fetal infection	*Reduced uteroplacental perfusion*
Rubella	Preeclampsia
CMV	Maternal hypertension
Toxoplasmosis	Diabetes mellitus
Malaria	Maternal smoking
Syphilis	
	Undernutrition
Chromosome abnormalities	Evidence of malnutrition
Trisomy 13, 18, 21	Adolescent mother
Triploidy	Chronic infection
Sex chromosome abnormalities	Alcoholism; drug ingestion
	Dietary faddism
Metabolic abnormalities	Maternal PKU
Agenesis of pancreas/absent islets of Langerhans	
Gangliosidosis I	
I cell disease	
Hypophosphatasia	
Menkes' syndrome	

CMV, cytomegalovirus; PKU, phenylketonuria.

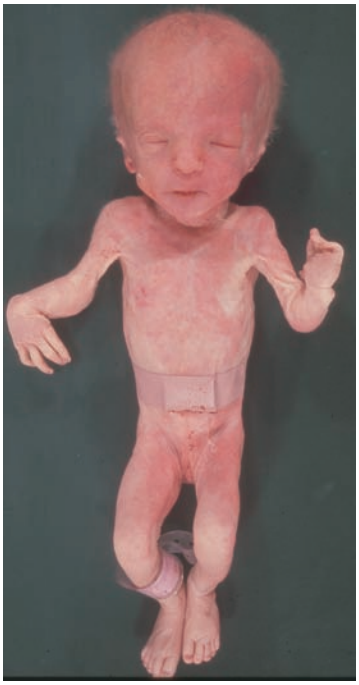


Figure 2.4. Asymmetric growth restriction; the head is relatively large, the trunk and limbs are thin, and flexion deformities are present, secondary to oligohydramnios.

ity of nutrients as seen in chronic maternal undernutrition. Prematurity and its related problems are discussed in Chapter 11.

Some babies are inappropriately large for gestation age (heavy-for-dates). These babies tend to be born to taller, heavier women who are older and of higher parity. Excessive maternal weight gain may be observed during pregnancy. Maternal diabetes mellitus (Fig. 2.5) and gestational diabetes are associated with large babies. Macrosomia is a feature of Wiedemann–Beckwith and Soto's syndromes. Large babies are at risk of birth injury and intrapartum hypoxia (see Chapter 13).

Equipment for Perinatal Necropsy

Necropsy examination of fetuses and neonates requires few facilities beyond those that one would expect to find in a well-equipped mortuary. Onsite radiographic and photographic facilities are essential. Any pathology department where examination of babies takes place frequently must have equipment that will allow easy, accurate recording of body measurement and organ weights.

Measuring

Accurate weighing scales for measurement of both body and organ weights are essential. Mature babies can usually be weighed on scales suitable for adult organ weighing. For organ weights and the weighing of fetuses less than 20 weeks' gestation, scales accurate to 0.5 g are needed. An electronic balance with digital display is very convenient.

A metric ruler and calipers or measuring board with one fixed end (Langley 1971) are required for measurement of body and foot lengths and skull diameters. Circumferential measurements are best made with string and read off against a ruler; a tape measure is less accurate. Techniques of mensuration are illustrated by Valdes-Dapena and Huff (1983).

Dissection Instruments

Instruments of appropriately small size make fetal dissection much easier to carry out without damage to structures before examination is complete. Scissors with tapering blades and rounded

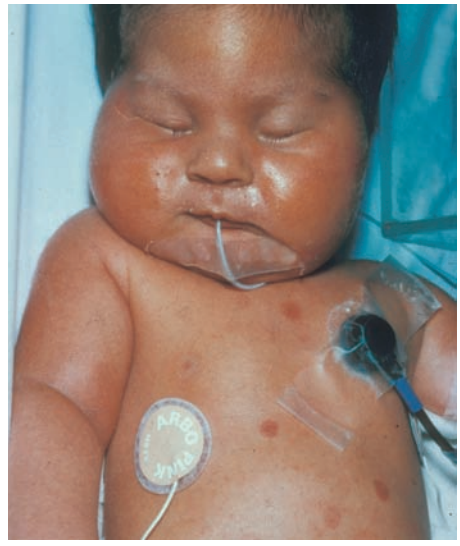


Figure 2.5. Infant of diabetic woman is both large and obese, birth weight 3614 g at 38 weeks' gestation (median birth weight, 2940 g). (Courtesy of Dr. A.R. Wilkinson, Oxford.)

ends (Mayo) reduce the frequency of inadvertent perforation, which often occurs when pointed scissors are used. Iridectomy scissors are a useful size for dissection of second trimester abortuses. Non-toothed forceps are less damaging to fetal tissues than those with teeth. A range of models is available and a selection of round-ended, tapering-blade forceps of different lengths, such as 10 to 17 cm (4–7 inches), is useful.

Size 3 and 4 scalpel handles and a range of blades to fit will suit most purposes during fetal examination. Neck dissection in the neonate is a great deal easier with a narrow scalpel blade of small size.

A selection of probes, down to small lachrymal duct size, makes demonstration of abnormal anatomy easier. A pair of 20-cm (8-inch) straight, sprung bone forceps will cut ribs, vertebral pedicles, and femoral shafts, and open middle ears. A domestic cake slice is invaluable for handling brain slices.

A mounted magnifying lens with an integral light source is especially useful during the examination of second trimester fetuses. A dissecting microscope for detailed examination of very small hearts is recommended.

Postmortem Examination

The postmortem examination of fetuses and babies dying in the neonatal period follows a similar pattern to examination of adults but differs in certain important respects. Neglect of these modifications results in failure to obtain important information or causes unnecessary difficulty in the performance of necropsy. It is better to adopt modifications of necropsy technique that are appropriate to the demonstration of a wide range of pathological and developmental abnormalities and to use them for all perinatal necropsies than to use a variety of minor modifications of technique when a particular abnormality is suspected. This promotes familiarity with the techniques adopted and means that abnormalities that were not diagnosed during life are less likely to be missed.

Perinatal necropsy protocols are detailed by Langley (1971), Barson (1981), Pryse-Davies (1981), Wigglesworth (1984), and Chambers (1992). Guidelines summarizing essential investigations are published by the Royal College of Pathologists (1993) and the College of American Pathologists (Bove 1997).

Measurement

Body weight and external measurements are recorded and compared with standard weight charts. The best comparisons are data sets compiled from the local population (Fig. 2.6), but such data are not often available. Standards used by local clinicians are the best compromise. Charts based on data of Gairdner and Pearson (1971) and Milner and Richards (1974) are available commercially. It is important that weights and measurements are assessed against appropriate gestation-related normal values. The frequency of growth restriction among babies who die in the perinatal period can influence the pathologist's concept of normality to the extent that significant growth restriction may be ignored. Crown–rump, crown–heel, and foot lengths are useful for assessment of fetal growth. Foot length (Table 2.2) is a useful gestation-related measurement to supplement menstrual dating and for comparison with ultrasound measurements made during pregnancy or clinical assessment of maturity undertaken at birth. Both crown–rump and crown–heel lengths are prone to inaccuracy, particularly following intrauterine death, when joint ligaments are lax and permit excessive stretching. Foot length has the advantage that it is less open to manipulation of this type, but its range is small and this measurement should be carefully performed.

Information about other measurements at different gestations, such as head and abdominal circumference (Campbell and Pearce 1985), BPD, and femoral length (Yagel et al. 1986) has become available as a result of prenatal ultrasound fetal measurement.

Head circumference should be recorded. It is approximately equal to crown–rump length during the second and third trimesters of pregnancy (Fig. 2.7). It provides a useful indication of the appropriateness of head size and may also draw attention to growth restriction.

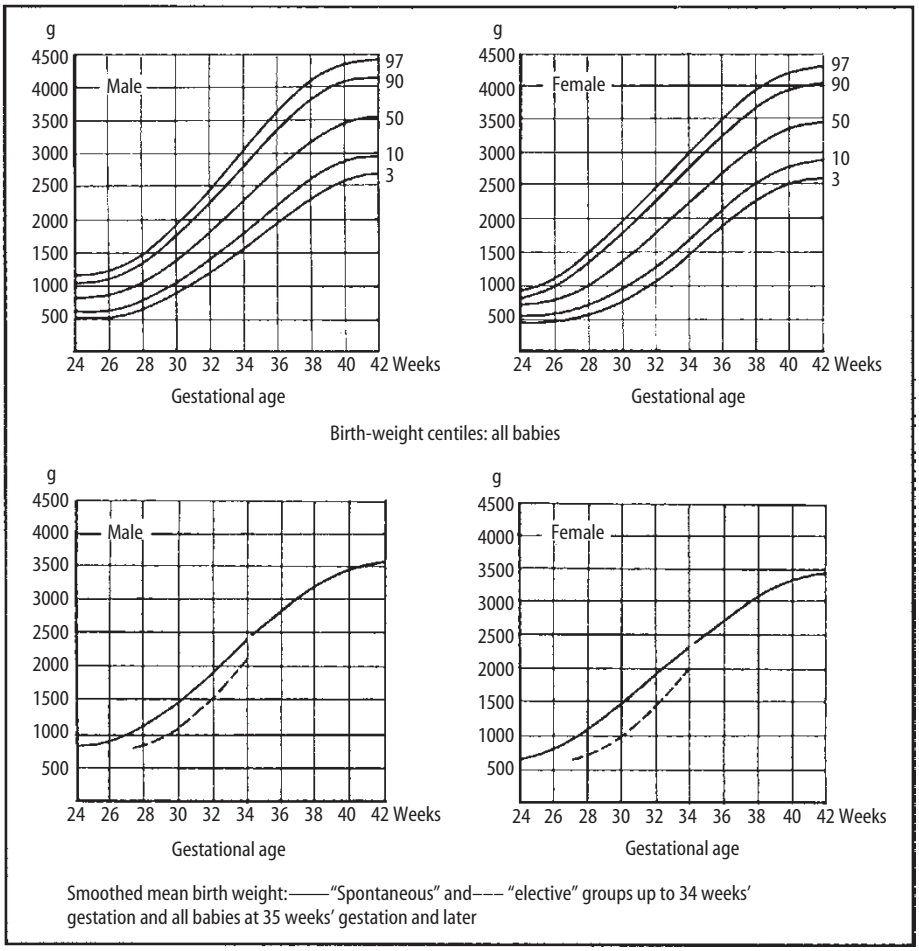


FIGURE 2.6. Smoothed percentiles of birth weight by sex and gestation (Yudkin et al. 1987).

Postmortem Imaging

A whole-body radiograph is an important adjunct to perinatal necropsy (Russell 1981) and mandatory in the investigation of generalized skeletal disorders and skeletal deformity (Kalifa et al. 1989).

Access to the radiography department for postmortem radiographs is often difficult. An appropriate apparatus sited within the pathology department usually means that radiographs are performed regularly. A self-contained unit, such as the Faxiton (Hewlett-Packard) is ideal for pathologists' use, and gives the best results for fetal radiography and examination of individual bones (Kjaer et al. 1991). Very good results can be obtained with mammography or fine grain industrial film.

An anteroposterior radiograph of the whole body will detect disproportion between trunk and limbs, and between the different components of limbs, more reliably than visual assessment. It can also provide information pertaining to maturity (Table 2.3; Russell 1981). The appearance of ossification centers is not sufficiently reliable to be used to assess gestational age but permits examination of the relationship between skeletal development and other parameters of gestation. Some of the factors that affect the appearance of ossification centers are discussed by Pryse-Davies et al.

TABLE 2.2. Fetal autopsy standards at 12 to 27 weeks of gestation (Maroun and Graem 2005)

Age		FL (mm)	CR (cm)	CH (cm)	HDC (cm)	Body (g)	Brain (g)	Liver (g)			Lungs (g)		Heart (g)	Thymus (g)			Spleen (g)		Kidneys (g)		Adrenals (g)	
(GW)	Maceration[a]	0–3	0–3	0–3	0–3	0–3	0–3	0–1	2	3	0–1	2–3	0–3	0–1	2	3	0–1	2–3	0–1	2–3	0–1	2–3
12	Mean	9	7.4	9.8	7.1	29.6	4.8	1.5	1.4	1.3	0.6	0.9	0.10	0.03			0.01		0.25	0.19	0.04	0.11
	SD	*3*	*1.1*	*1.7*	*1.1*	*14.9*	*1.4*	*1.2*	*1.2*	*1.2*	*0.9*	*0.9*	*0.14*	*0.06*			*0.02*		*0.15*	*0.15*	*0.18*	*0.18*
13	Mean	12	8.7	11.8	8.5	37.4	6.5	2.0	1.7	1.7	1.2	1.2	0.20	0.04			0.02	0.08	0.3	0.2	0.17	0.17
	SD	*3*	*1.2*	*1.8*	*1.2*	*14.9*	*1.4*	*1.2*	*1.2*	*1.2*	*0.9*	*0.9*	*0.14*	*0.06*			*0.03*	*0.03*	*0.1*	*0.1*	*0.18*	*0.18*
14	Mean	15	9.9	13.7	9.8	53.0	9.1	2.9	2.4	2.3	2.0	1.5	0.3	0.05	0.07	0.05	0.04	0.14	0.4	0.3	0.3	0.2
	SD	*3*	*1.2*	*1.8*	*1.2*	*14.9*	*2.5*	*1.2*	*1.2*	*1.2*	*0.9*	*0.9*	*0.1*	*0.06*	*0.06*	*0.06*	*0.04*	*0.04*	*0.1*	*0.1*	*0.2*	*0.2*
15	Mean	18	11.1	15.6	11.1	76.5	12.7	4.2	3.3	3.2	2.9	2.1	0.5	0.07	0.08	0.06	0.06	0.17	0.6	0.5	0.5	0.3
	SD	*3*	*1.2*	*1.8*	*1.2*	*18.5*	*3.9*	*1.2*	*1.2*	*1.2*	*0.9*	*0.9*	*0.1*	*0.06*	*0.06*	*0.06*	*0.06*	*0.06*	*0.3*	*0.3*	*0.2*	*0.2*
16	Mean	21	12.4	17.5	12.4	108	17.3	5.9	4.5	4.2	3.9	2.7	0.8	0.11	0.12	0.09	0.09	0.17	0.9	0.8	0.6	0.4
	SD	*3*	*1.3*	*1.8*	*1.3*	*41*	*5.4*	*1.5*	*1.5*	*1.5*	*1.2*	*1.2*	*0.2*	*0.06*	*0.06*	*0.06*	*0.08*	*0.08*	*0.4*	*0.4*	*0.3*	*0.3*
17	Mean	24	13.5	19.3	13.6	147	22.9	8.1	6.1	5.4	5.1	3.5	1.0	0.18	0.18	0.12	0.13	0.16	1.3	1.1	0.8	0.5
	SD	*3*	*1.3*	*1.9*	*1.3*	*53*	*6.9*	*3.0*	*3.0*	*3.0*	*1.7*	*1.7*	*0.4*	*0.06*	*0.06*	*0.06*	*0.12*	*0.12*	*0.6*	*0.6*	*0.4*	*0.4*
18	Mean	27	14.7	21.1	14.8	194	29.4	10.7	7.9	6.8	6.4	4.4	1.4	0.3	0.3	0.2	0.19	0.15	1.8	1.5	1.0	0.7
	SD	*3*	*1.3*	*1.9*	*1.3*	*65*	*8.4*	*4.5*	*4.5*	*4.5*	*2.3*	*2.3*	*0.5*	*0.2*	*0.2*	*0.2*	*0.17*	*0.17*	*0.8*	*0.8*	*0.4*	*0.4*
19	Mean	30	15.9	22.9	16.0	249	37.0	13.8	10.1	8.4	7.9	5.4	1.7	0.4	0.4	0.3	0.3	0.15	2.4	2.0	1.2	0.8
	SD	*3*	*1.3*	*1.9*	*1.3*	*78*	*9.8*	*6.0*	*6.0*	*6.0*	*2.8*	*2.8*	*0.7*	*0.3*	*0.3*	*0.3*	*0.2*	*0.22*	*1.0*	*1.0*	*0.5*	*0.5*
20	Mean	33	17.0	24.6	17.2	312	45.5	17.2	12.5	10.2	9.5	6.5	2.1	0.6	0.5	0.3	0.4	0.17	3.0	2.5	1.4	1.0
	SD	*3*	*1.4*	*1.9*	*1.4*	*92*	*11.3*	*7.5*	*7.5*	*7.5*	*3.4*	*3.4*	*0.8*	*0.4*	*0.4*	*0.4*	*0.3*	*0.29*	*1.2*	*1.2*	*0.6*	*0.6*
21	Mean	36	18.2	26.3	18.3	382	55.0	21.1	15.2	12.3	11.2	7.8	2.6	0.8	0.7	0.4	0.5	0.22	3.8	3.1	1.7	1.2
	SD	*3*	*1.4*	*2.0*	*1.4*	*107*	*12.8*	*9.0*	*9.0*	*9.0*	*4.0*	*4.0*	*1.0*	*0.5*	*0.5*	*0.5*	*0.4*	*0.36*	*1.4*	*1.4*	*0.7*	*0.7*
22	Mean	39	19.3	28.0	19.4	461	65.4	25.5	18.2	14.5	13.1	9.2	3.1	1.0	0.9	0.6	0.7	0.3	4.6	3.8	1.9	1.4
	SD	*3*	*1.4*	*2.0*	*1.4*	*122*	*14.3*	*10.4*	*10.4*	*10.4*	*4.6*	*4.6*	*1.1*	*0.6*	*0.6*	*0.6*	*0.4*	*0.4*	*1.6*	*1.6*	*0.8*	*0.8*
23	Mean	41	20.4	29.6	20.5	547	76.9	30.2	21.6	16.9	15.1	10.7	3.6	1.3	1.1	0.7	0.9	0.4	5.5	4.6	2.2	1.6
	SD	*4*	*1.5*	*2.0*	*1.4*	*122*	*15.8*	*11.9*	*11.9*	*11.9*	*5.3*	*5.3*	*1.3*	*0.8*	*0.8*	*0.8*	*0.5*	*0.5*	*1.9*	*1.9*	*0.8*	*0.8*
24	Mean	44	21.5	31.2	21.6	641	89.3	35.4	25.2	19.5	17.3	12.4	4.2	1.6	1.3	0.8	1.1	0.6	6.5	5.5	2.5	1.8
	SD	*4*	*1.5*	*2.0*	*1.5*	*137*	*17.2*	*13.4*	*13.4*	*13.4*	*5.9*	*5.9*	*1.4*	*0.9*	*0.9*	*0.9*	*0.6*	*0.6*	*2.1*	*2.1*	*0.9*	*0.9*
25	Mean	47	22.6	32.8	22.6	743	103	41.1	29.1	22.3	19.6	14.1	4.9	1.9	1.6	1.0	1.4	0.8	7.6	6.4	2.8	2.0
	SD	*4*	*1.5*	*2.1*	*1.5*	*154*	*19*	*14.9*	*14.9*	*14.9*	*6.6*	*6.6*	*1.6*	*1.1*	*1.1*	*1.1*	*0.7*	*0.7*	*2.4*	*2.4*	*1.0*	*1.0*
26	Mean	50	23.6	34.3	23.6	853	117	47.1	33.4	25.3	22.0	16.0	5.6	2.3	1.9	1.2	1.7	1.1	8.8	7.4	3.1	2.3
	SD	*4*	*1.5*	*2.1*	*1.5*	*171*	*20*	*16.4*	*16.4*	*16.4*	*7.3*	*7.3*	*1.7*	*1.2*	*1.2*	*1.2*	*0.9*	*0.9*	*2.7*	*2.7*	*1.1*	*1.1*
27	Mean	52	24.7	35.8	24.5	971	133	53.6	37.9	28.6	24.6	18.0	6.3	2.6	2.2	1.4	2.1	1.4	10.1	8.4	3.4	2.5
	SD	*4*	*1.6*	*2.1*	*1.5*	*188*	*22*	*17.9*	*17.9*	*17.9*	*8.0*	*8.0*	*1.8*	*1.4*	*1.4*	*1.4*	*1.0*	*1.0*	*3.0*	*3.0*	*1.2*	*1.2*

CH, crown-heel length; CR, crown-rump length; FL, foot length; GW, weeks of gestation; HDC, head circumference; SD, standard deviation.

[a]0 = none, 1 = mild, 2 = moderate, 3 = marked.

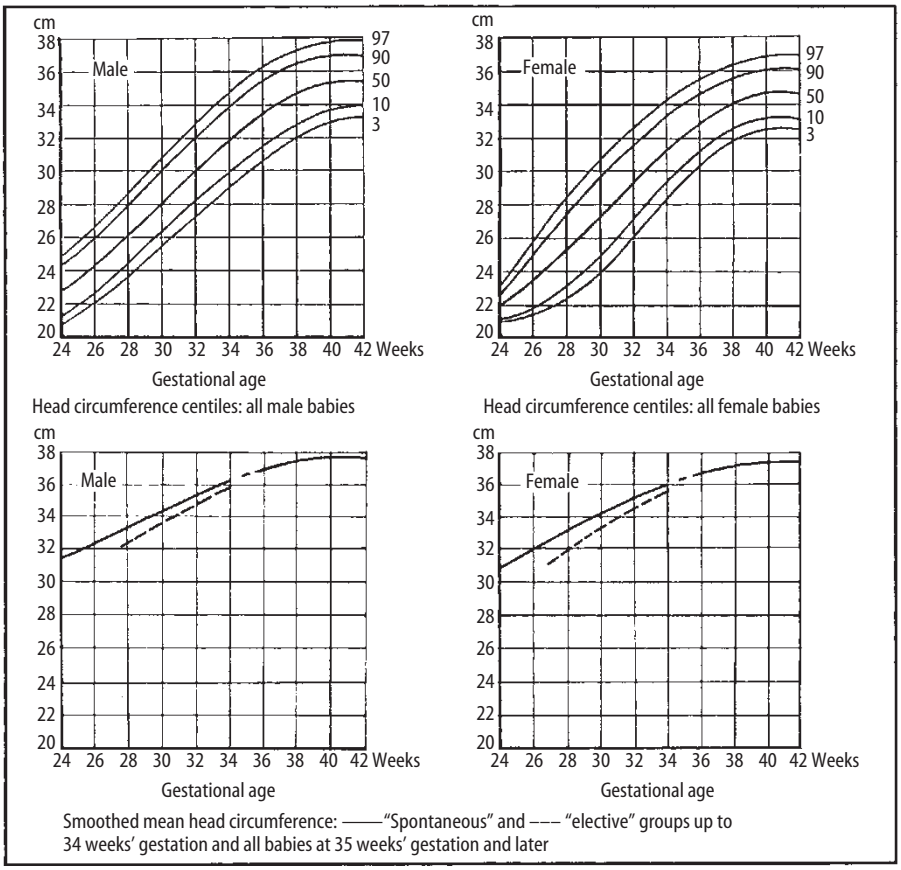


Figure 2.7. Centiles of head circumference by sex and gestational age (Yudkin et al. 1987).

(1974). Measurement of long bones and the thoracic and lumbar spine is useful for evaluating gestation from about 13 weeks to term (van der Harten et al. 1990). A more sophisticated method of assessment of bone age using parameters previously omitted from assessment, including detailed mineralization of teeth and changes in shape in centers of ossification as well as the mere presence or absence of a center, has been found to be a reliable guide to gestational age in the second half of pregnancy (Stempflé et al. 1995). A radiograph will detect local skeletal abnormalities (Fig. 2.8), extraskeletal calcification, air embolism, and abnormal accumulations of gas such as pneumopericardium (Fig. 2.9).

Table 2.3. Times of appearance of bone centers on postnatal radiograph film (derived from Russell 1981)

Calcaneus	25 weeks
Talus	27 weeks
Lower femoral center	36 weeks
Upper tibial center	37 weeks

Contrast studies provide a useful record of some malformations (Foote et al. 1978; Hawass et al. 1989) and may be valuable for direction of subsequent dissection (Fig. 2.10).

Postmortem radiography has an established role in perinatal postmortem examination. Bourliere-Najean et al. (2003) found radiography of most use in fetuses examined following termination for fetal anomaly. Now other imaging techniques have been introduced. Ultrasound scans can demonstrate a variety of malformations (Furness et al. 1989). Magnetic resonance imaging contributes to the evaluation of intracranial pathology, particularly malformations (Brookes

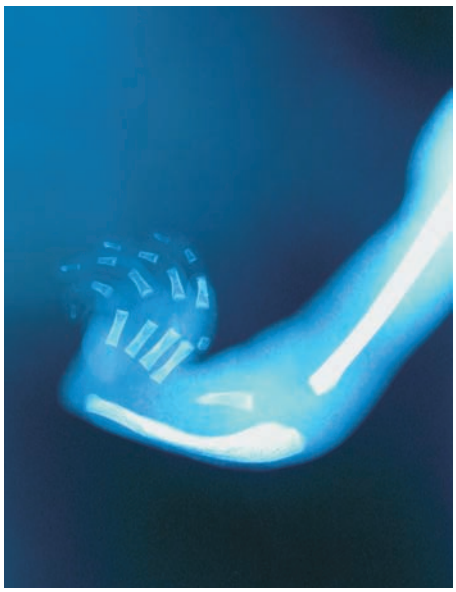


Figure 2.8. Postmortem radiograph, radial hypoplasia.

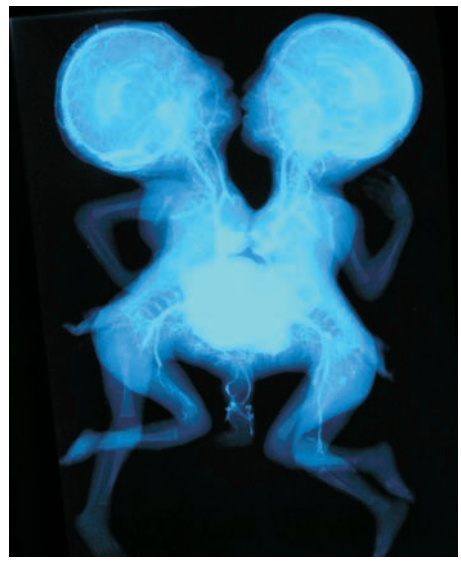


Figure 2.10. Contrast radiograph of conjoined twins at 20 weeks' gestation indicates sites of vascular connection.

et al. 1996; Woodward et al. 1997; Fig. 2.11) but it is important to make careful comparison with the morbid anatomical state. While the use of three-dimensional MRI can evaluate complex cardiac malformations, it is time-consuming (Meyer-Wittkopf et al. 1996); however, it might be useful when autopsy authorization is declined. The introduction of dedicated MRI systems into pathology departments could bridge the gap between prenatal investigations and postmortem findings, particularly when central nervous system (CNS) malformations are present (Langer et al. 1998).

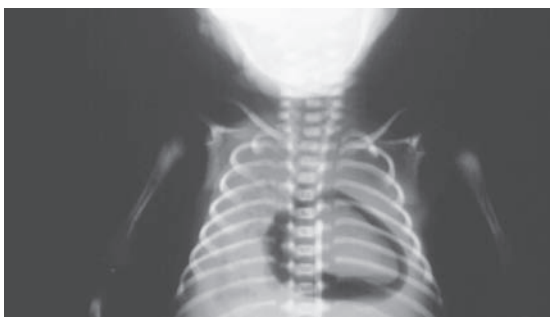


Figure 2.9. Postmortem radiograph, large pneumopericardium. (Courtesy of Dr. R. Nairn, Kilmarnock, Scotland.)

Photography

A photographic record of dysmorphic features and other external abnormalities provides both a quicker and a more accurate record than a lengthy

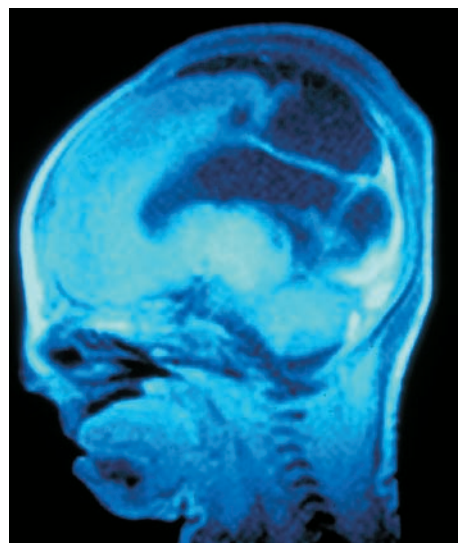


Figure 2.11. Postmortem magnetic resonance (MR) demonstrates cystic leukomalacia and ventriculomegaly. (Courtesy of Dr. M. McPhillips, Edinburgh.)

written description. Russell (1994) offers many practical suggestions about specimen photography. I prefer a glass support with colored (changeable) background to the black velvet he recommends. Such photographs are particularly useful when further opinion is sought in difficult cases. It is important to photograph the fetus from pregnancies terminated for malformation. Regular photographic recording of dysmorphic detail is a useful educational tool for the individual pathologist as well as being of enormous value for teaching trainees. Serial photographs during the course of organ dissection, often before the organs are removed from the body, are commonly required during fetal and neonatal examination. This need is best met by a mounted, high-definition digital camera and monitor together with adjustable lighting located within the mortuary. The equipment should be easy to use. Good recording of dysmorphic features results in a high proportion of firm diagnoses and better parental counseling, as well as more interesting clinical meetings and lectures.

External Examination

Dysmorphic features should be sought in an orderly fashion prior to dissection during the examination of all fetal and neonatal deaths. These features, taken in combination with major malformations, permit accurate syndrome diagnosis, which enables specific recurrence risks to be given to parents (see Chapter 6). Recognition of typical external abnormalities (Figs. 2.12 to 2.16) alerts the prosector to the likely type or range of internal malformations as well as giving an indication of the type of investigations such as chromosome culture or biochemical assay that might be necessary for a firm diagnosis to be reached.

The presence of other external abnormalities, including the site of drains or catheters or surgical incisions, should be noted. Evidence of injuries, related to delivery, prenatal investigations, or neonatal intensive care, should be carefully sought.

The appearance and color of the skin may give clues about time of death (maceration), maturity (bright pink in the very preterm baby, wrinkled and flaking in postmaturity). Extreme pallor might alert one to the possibility of acute hemorrhage. The presence of jaundice, bruising, or petechial hemorrhages that are not explained clinically require further investigation.

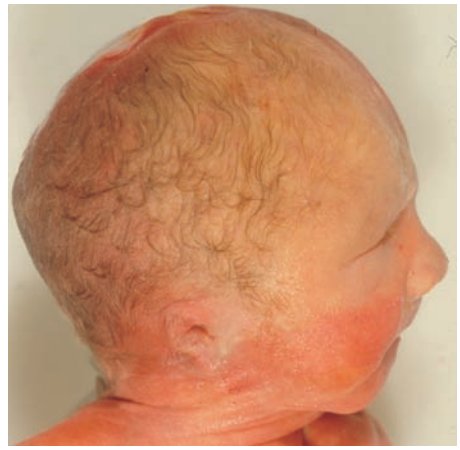


Figure 2.12. Trisomy 13: microcephaly, sloping forehead, micrognathia, and low-set ears with abnormal helix are shown.

Examination of Body Cavities

Before the body cavities are opened, it is important to look for pneumothorax, if a radiograph has not been done, in all neonatal deaths, even if survival has been very brief. This can be done by

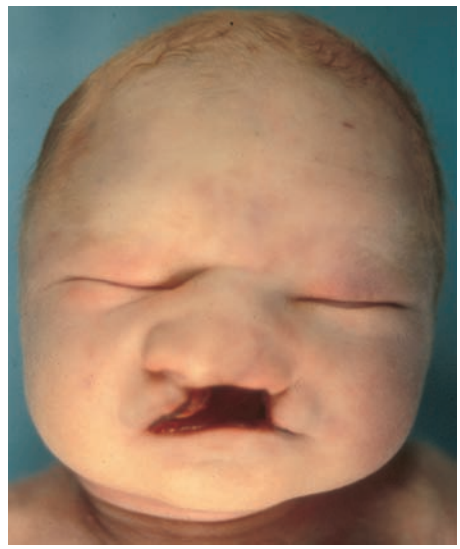


Figure 2.13. Trisomy 13: hypertelorism and bilateral cleft lip/palate.

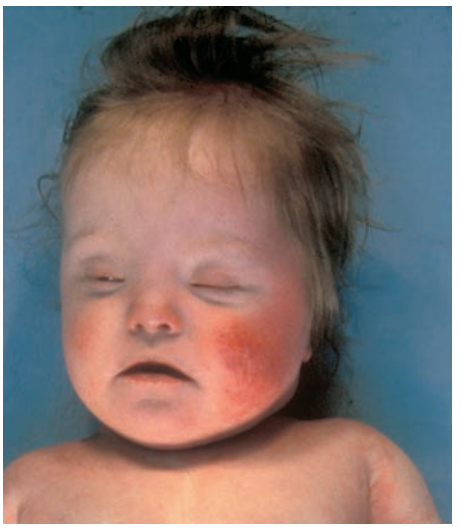
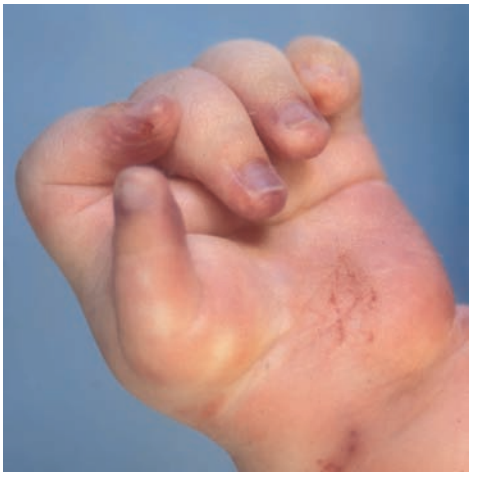
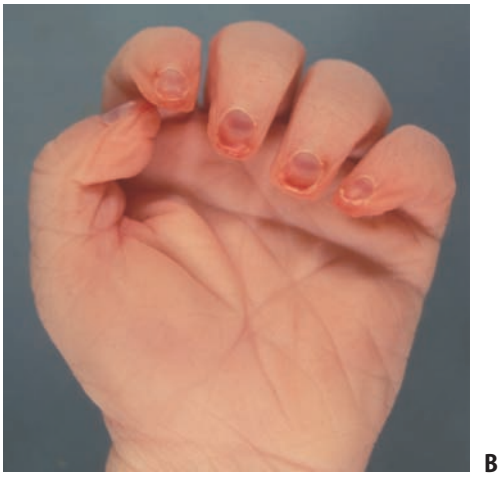


Figure 2.14. Trisomy 21: up-slanting palpebral fissures, inner epicanthal folds, small nose.



A



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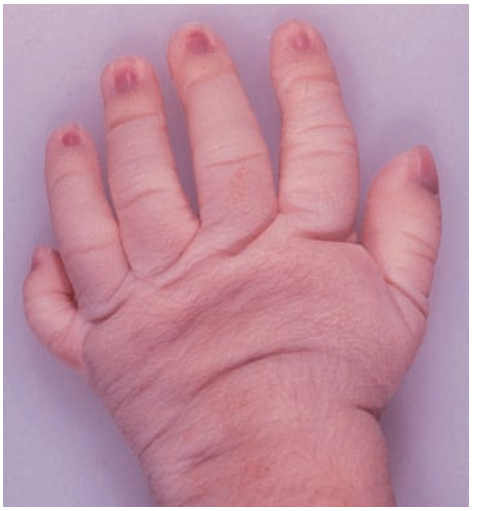

C

Figure 2.15. (A) Trisomy 18: overlapping fingers is common. (B) Trisomy 21: transverse palmar crease. (C) Meckel-Gruber syndrome: postaxial polydactyly.

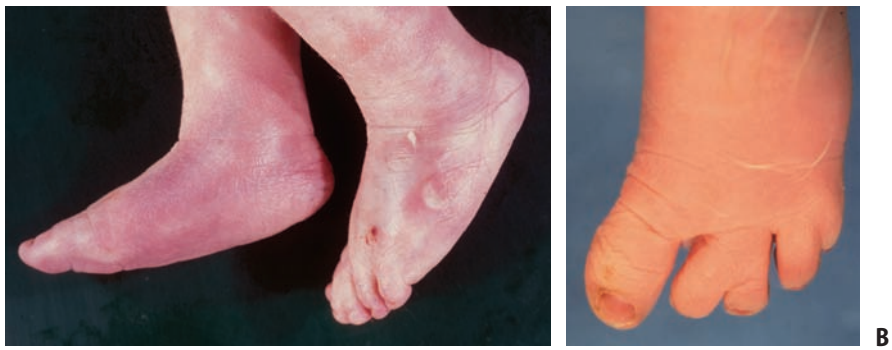

FIGURE 2.16. Trisomy 18. (A) Rocker-bottom feet. (B) Syndactyly of 2nd and 3rd toes.

immersing the baby in water and inserting a trocar and cannula into each hemithorax in turn. The sixth intercostal space in the midaxillary line is a convenient entry point. When a pneumothorax is present, a stream of gas bubbles will issue from the cannula when the trocar is removed.

The skin can be opened with a straight, midline incision skirting to the left of the umbilicus or with Y- or inverted Y-shaped incisions. A Y-shaped incision is practical when viewing of the baby is likely after necropsy. An inverted Y allows forward reflection of the bladder and umbilical vessels, and one limb of the Y can be continued down the thigh to the level of the knee medial to the patella to facilitate removal of the femur.

Skin and subcutaneous tissues are dissected off the rib cage, taking note of the amount of subcutaneous fat and the color and volume of muscle, which may be pale and reduced in neuromuscular disorders. The abdominal viscera are examined in situ. The presence of ascites, blood, pus, or fecal material in the cavity is sought, and any adhesions between viscera are noted. Intestinal rotation and the presence of hernias, volvulus, or intussusception are noted. The size, position, and contour of the liver are inspected, and its color, surface appearance, and presence of hematomas are recorded.

The sternum is removed either by cutting through costal cartilages about 5 mm medial to the costochondral junction or cutting through the ribs in the anterior axillary line. Either method ensures that costochondral junctions are intact for subsequent histological examination. Dividing the ribs gives wide access to the thoracic cavity, facilitating inspection and photography of thoracic viscera in situ.

The relative size of heart, lungs, and thoracic diameter are observed, and the presence of pleural and pericardial effusions is sought. The level and completeness of the diaphragm are noted. The route of chest drains is explored, looking particularly at the position of the drain tip and searching for visceral injury, particularly in the presence of hemothorax.

The thymus lies in the upper part of the anterior mediastinum. It is bilobed with narrow extensions up through the thoracic inlet into the neck. It is a large organ weighing 12 to 18 g at term but is relatively smaller in preterm infants. When of normal size, it overlies the aorta and pulmonary trunk. Petechial hemorrhages within it are often present following acute antepartum or intrapartum hypoxia, particularly when there is retroplacental hemorrhage. Its size is much reduced following long-standing stress of any sort. It is convenient to dissect the thymus off the pericardium at this point, taking care not to damage the innominate vein, which runs behind it at the thoracic inlet. The thymus is usually absent in DiGeorge syndrome, although a nodule of thymic tissue may be found on the lateral border of the pericardial sac in that condition. The thymus of the sick preterm baby who dies in the late neonatal period is often very small and easily overlooked. This situation should not be confused with DiGeorge syndrome. If in doubt, reexamine the face; a backward sloping forehead and small nose are characteristic of DiGeorge syndrome.

Incise the pericardium in the midline, noting the appearance and amount of any effusion or hemorrhage. The exterior of the heart and its vascular connections should be examined in situ.

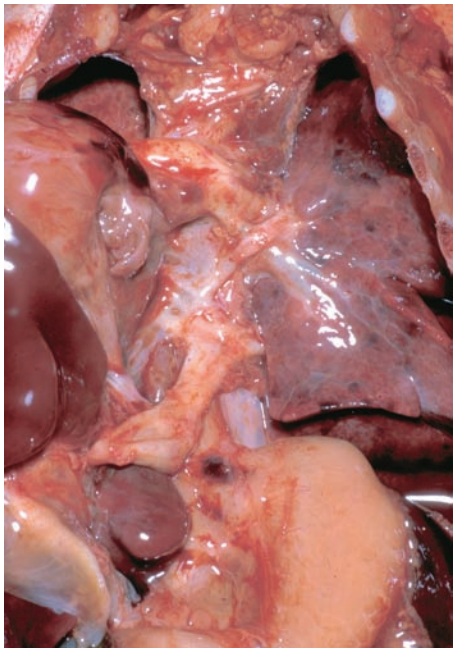

Figure 2.17. Traction of the heart to the right shows the confluence of pulmonary veins; an anomalous venous trunk runs down through the diaphragm.

Note the shape and size of the atria and their appendage to determine atrial situs. The right atrium is dilated in heart failure and when there is anomalous pulmonary venous return. It may be small when the tricuspid and pulmonary valves are stenosed or atretic. The left atrium is small in mitral atresia or stenosis, or in the presence of pulmonary hypoplasia or total anomalous pulmonary venous return.

Inspect the distribution of the coronary arteries. The anterior descending branch of the left coronary artery is the surface marking of the interventricular septum. Its position indicates the relative contribution of each ventricle to the ventricular mass. At term the right ventricle is relatively larger and its wall relatively thicker than in later life. Abnormal coronary artery distribution, particularly a leash of small vessels running obliquely across the anterior surface of the ventricles, should alert one to the possibility of a defective interventricular septum or abnormalities of the origin or relationships of great arteries. The relative size and position of the aorta and pulmonary artery are noted; they should be of approximately equal size, with the pulmonary artery crossing in front of the aorta. The distribution of the aortic arch branches and configuration of the superior vena cava and its tributaries are noted. The heart should be pulled to the right; observe the course of the left pulmonary veins, normally running to the back of the left atrium. Where pulmonary venous drainage is abnormal, the pulmonary veins join to form a trunk that may ascend to join a superior vena cava or downward through the diaphragm to join the portal venous system (Fig. 2.17) or, occasionally the inferior vena cava.

Evisceration

At this point, the prosector has a number of options for evisceration of the body. One of the important differences between necropsy examination of babies and adults is that organs should not be removed individually but rather in continuity as one, two, or three large blocks. Barson (1981) and Valdes-Dapena and Huff (1983) advocate the removal of the thoracic and abdominal organs together, beginning with separation of neck structures after bringing down the tongue; midline thoracic structures are freed from prevertebral fascia; the diaphragm is cut through and all the abdominal viscera removed in continuity with thoracic organs, cutting through the bladder neck and rectum to complete removal. The viscera are then dissected from the back. I adopt this method in those cases where there are pathological abnormalities of the intestines, particularly if genitourinary abnormalities are also present; however, it is less satisfactory for cardiac examination.

Pryse-Davies (1981) and Wigglesworth (1984) advocate routine removal of viscera in four blocks: neck structures and thoracic viscera, small and large intestines, upper abdominal viscera, and urogenital tracts and related vessels. I favor the removal of thoracic and upper abdominal viscera in one block for all perinatal necropsies as advocated by Langley (1971) and by Wigglesworth (1984) for cases where congenital heart disease is suspected. Adoption of this method as a routine reduces the number of decisions that need to be made during dissection and therefore reduces error. It also means that the prosectors become familiar with a technique more quickly, and if they have failed to notice a hint in the clinical notes or forgotten to look at the pulmonary veins

TABLE 2.4. Fetal autopsy standards at 28 to 43 weeks of gestation (Maroun and Graem 2005)

Age		FL (mm)	CR (cm)	CH (cm)	HDC (cm)	Body (g)	Brain (g)	Liver (g)			Lungs (g)		Heart (g)	Thymus (g)			Spleen (g)		Kidneys (g)		Adrenals (g)	
(GW)	Maceration[a]	0–3	0–3	0–3	0–3	0–3	0–3	0–1	2	3	0–1	2–3	0–3	0–1	2	3	0–1	2–3	0–1	2–3	0–1	2–3
28	Mean	55	25.7	37.3	25.5	1096	149	60.6	42.7	32.0	27.4	20.2	7.1	3.1	2.5	1.6	2.5	1.8	11.4	9.6	3.7	2.8
	SD	*4*	*1.6*	*2.2*	*1.6*	*206*	*23*	*19.3*	*19.3*	*19.3*	*8.7*	*8.7*	*2.0*	*1.6*	*1.6*	*1.6*	*1.1*	*1.1*	*3.3*	*3.3*	*1.3*	*1.3*
29	Mean	57	26.7	38.7	26.4	1230	166	67.9	47.8	35.6	30.2	22.5	7.9	3.5	2.9	1.8	3.0	2.2	12.9	10.8	4.1	3.1
	SD	*4*	*1.6*	*2.2*	*1.6*	*225*	*25*	*20.8*	*20.8*	*20.8*	*9.5*	*9.5*	*2.1*	*1.8*	*1.8*	*1.8*	*1.3*	*1.3*	*3.6*	*3.6*	*1.4*	*1.4*
30	Mean	60	27.7	40.1	27.2	1371	185	75.7	53.3	39.4	33.2	24.9	8.7	4.0	3.3	2.1	3.6	2.7	14.4	12.1	4.5	3.4
	SD	*4*	*1.6*	*2.2*	*1.6*	*244*	*26*	*22.3*	*22.3*	*22.3*	*10.2*	*10.2*	*2.3*	*2.1*	*2.1*	*2.1*	*1.4*	*1.4*	*3.9*	*3.9*	*1.4*	*1.4*
31	Mean	62	28.7	41.4	28.1	1520	204	83.9	59.0	43.4	36.3	27.4	9.6	4.5	3.7	2.3	4.2	3.3	16.0	13.4	4.8	3.8
	SD	*4*	*1.7*	*2.2*	*1.7*	*264*	*28*	*23.8*	*23.8*	*23.8*	*11.0*	*11.0*	*2.4*	*2.3*	*2.3*	*2.3*	*1.6*	*1.6*	*4.3*	*4.3*	*1.5*	*1.5*
32	Mean	64	29.7	42.8	28.9	1677	224	92.6	65.0	47.6	39.6	30.0	10.6	5.0	4.2	2.6	4.8	3.9	17.7	14.9	5.2	4.1
	SD	*4*	*1.7*	*2.3*	*1.7*	*285*	*29*	*25.3*	*25.3*	*25.3*	*11.8*	*11.8*	*2.6*	*2.5*	*2.5*	*2.5*	*1.8*	*1.8*	*4.6*	*4.6*	*1.6*	*1.6*
33	Mean	67	30.6	44.0	29.7	1842	245	102	71.3	52.1	43.0	32.8	11.6	5.6	4.6	2.9	5.5	4.5	19.5	16.4	5.6	4.5
	SD	*4*	*1.7*	*2.3*	*1.7*	*306*	*31*	*27*	*26.7*	*26.7*	*12.6*	*12.6*	*2.7*	*2.8*	*2.8*	*2.8*	*1.9*	*1.9*	*5.0*	*5.0*	*1.7*	*1.7*
34	Mean	69	31.6	45.3	30.5	2015	268	111	77.9	56.7	46.6	35.7	12.6	6.2	5.1	3.2	6.3	5.2	21.4	18.0	6.0	4.8
	SD	*4*	*1.8*	*2.3*	*1.7*	*328*	*32*	*28*	*28.2*	*28.2*	*13.5*	*13.5*	*2.9*	*3.1*	*3.1*	*3.1*	*2.1*	*2.1*	*5.4*	*5.4*	*1.8*	*1.8*
35	Mean	71	32.5	46.5	31.2	2195	291	121	84.8	61.5	50.3	38.7	13.7	6.9	5.7	3.5	7.2	6.0	23.3	19.6	6.5	5.2
	SD	*5*	*1.8*	*2.3*	*1.8*	*350*	*33*	*30*	*29.7*	*29.7*	*14.3*	*14.3*	*3.0*	*3.3*	*3.3*	*3.3*	*2.3*	*2.3*	*5.8*	*5.8*	*1.9*	*1.9*
36	Mean	73	33.4	47.7	31.9	2383	315	132	92.1	66.5	54.1	41.9	14.8	7.5	6.2	3.8	8.1	6.7	25.4	21.4	6.9	5.6
	SD	*5*	*1.8*	*2.4*	*1.8*	*373*	*35*	*31*	*31.2*	*31.2*	*15.2*	*15.2*	*3.2*	*3.6*	*3.6*	*3.6*	*2.5*	*2.5*	*6.2*	*6.2*	*2.0*	*2.0*
37	Mean	76	34.3	48.9	32.6	2580	340	142	100	71.7	58.1	45.1	16.0	8.2	6.8	4.2	9.1	7.5	27.5	23.2	7.4	6.0
	SD	*5*	*1.8*	*2.4*	*1.8*	*397*	*36*	*33*	*33*	*32.7*	*16.1*	*16.1*	*3.3*	*3.9*	*3.9*	*3.9*	*2.7*	*2.7*	*6.6*	*6.6*	*2.1*	*2.1*
38	Mean	78	35.2	50.0	33.2	2784	366	154	107	77.2	62.2	48.5	17.2	8.9	7.4	3.9	10.1	8.3	29.8	25.0	7.8	6.5
	SD	*5*	*1.9*	*2.4*	*1.8*	*421*	*38*	*34*	*34*	*34.2*	*17.0*	*17.0*	*3.4*	*4.2*	*4.2*	*4.2*	*3.0*	*3.0*	*7.1*	*7.1*	*2.2*	*2.2*
39	Mean	82	37.0	52.1	34.4	3215	422	177	124	88.6	70.9	55.7	19.8	10.5	8.6	5.4	12.4	9.9	34.5	29.0	8.8	7.4
	SD	*5*	*1.9*	*2.4*	*1.9*	*446*	*39*	*36*	*36*	*35.6*	*18.0*	*18.0*	*3.6*	*4.6*	*4.6*	*4.6*	*3.2*	*3.2*	*7.5*	*7.5*	*2.3*	*2.3*
40	Mean	82	37.0	52.1	34.4	3215	422	177	124	88.6	70.9	55.7	19.8	10.5	8.6	5.4	12.4	9.9	34.5	29.0	8.8	7.4
	SD	*5*	*1.9*	*2.5*	*1.9*	*497*	*42*	*39*	*39*	*38.6*	*19.9*	*19.9*	*3.9*	*5.3*	*5.3*	*5.3*	*3.7*	*3.7*	*8.4*	*8.4*	*2.5*	*2.5*
41	Mean	84	37.8	53.1	35.0	3443	451	190	133	94.6	75.4	59.5	21.2	11.3	9.3	5.8	13.7	10.7	37.0	31.1	9.3	7.9
	SD	*5*	*1.9*	*2.5*	*1.9*	*497*	*42*	*39*	*39*	*38.6*	*19.9*	*19.9*	*3.9*	*5.3*	*5.3*	*5.3*	*3.7*	*3.7*	*8.4*	*8.4*	*2.5*	*2.5*
42	Mean	86	38.6	54.1	35.5	3678	481	203	142	101	80.1	63.4	22.5	12.2	10.0	6.2	15.0	11.5	39.6	33.3	9.9	8.4
	SD	*5*	*2.0*	*2.5*	*2.0*	*524*	*44*	*40*	*40*	*40*	*20.9*	*20.9*	*4.0*	*5.6*	*5.6*	*5.6*	*4.0*	*4.0*	*8.9*	*8.9*	*2.6*	*2.6*
43	Mean	88	39.4	55.0	36.0	3922	512	216	151	107	84.9	67.4	24.0	13.1	10.7	6.6	16.4	12.2	42.2	35.5	10.4	8.9
	SD	*5*	*2.0*	*2.5*	*2.0*	*551*	*45*	*42*	*42*	*42*	*21.9*	*21.9*	*4.2*	*6.0*	*6.0*	*6.0*	*4.2*	*4.2*	*9.4*	*9.4*	*2.7*	*2.7*

CH, crown-heel length; CR, crown-rump length; FL, foot length; GW, weeks of gestation; HDC, head circumference; SD, standard deviation.

[a] 0 = none, 1 = mild, 2 = moderate, 3 = marked.

earlier, then all is not lost. It also enables one to open the esophagus in continuity with the stomach, an opportunity that is lost with four-block evisceration.

Individual organs are weighed after dissection. Weights of fetal organs grouped by body weight and gestation are detailed in Tables 2.2 and 2.4.

Abdominal Vessels and Genitourinary System

The abdominal parts of the inferior vena cava and ascending aorta are opened in situ. It is convenient to leave aortic catheters in position until that vessel has been opened. The position of the catheter tip and any thrombus or endothelial damage is noted; renal vessels are opened. When there is no evidence of urinary tract obstruction, the adrenals and kidneys are dissected individually and weighed. The internal genitalia are inspected. If the scrotum is empty, testes are sought in the abdomen or inguinal canal.

Lower urinary tract obstruction usually occurs in males. Should lower urinary tract obstruction be evident, then the whole urinary tract should be removed in continuity (Fig. 2.18). The pubic symphysis is divided with strong scissors. Kidneys, ureters, and bladder, with aorta and iliac vessels, are freed from the top. The bladder neck is freed and the rectum divided just above the anus. The urethra is freed around its perimeter and pulled upward so that the penis is progressively invaginated using blunt dissection in a subcutaneous plane. When invagination is complete, the urethra is divided immediately proximal to its external meatus.

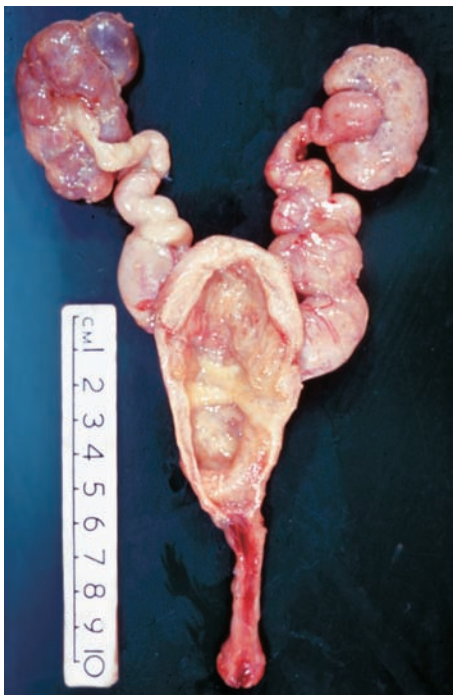

Figure 2.18. Lower urinary tract obstruction (posterior urethral valves). The urinary tract has been removed in continuity, the urethra opened from below, and the anterior bladder/prostatic urethral wall excised.

Thoracic and Upper Abdominal Viscera

Dissection of the thoracoabdominal block is begun from the back with organs resting on a sponge to reduce their mobility. The spleen is dissected free and weighed and the pancreas identified. Gross evidence of pancreatic involvement in mucoviscidosis is unusual in the neonate, and part of the pancreas should always be removed for histological examination. The esophagus is opened along its posterior wall and the stomach, around the greater curvature, then through the pylorus and duodenum. Esophageal atresia will be obvious in the early part of this maneuver (see Fig. 18.4B in Chapter 18). The contents of viscera are noted. Meconium may be present in esophagus and stomach following birth asphyxia, and gastric ulceration is sometimes seen following tolazoline medication (see Fig. 17.17 in Chapter 17). In duodenal atresia the relationship of the head of the pancreas to the duodenum should be carefully observed. Continuity of the bile ducts and appearance of the gallbladder are noted and the esophagus and stomach dissected off remaining viscera.

The larynx and trachea are opened down the posterior wall and their contents noted. The larynx should be carefully opened with forceps, and the presence and site of ulceration or fibrous scarring noted. Laryngeal atresia is an occasional

cause of respiratory distress at birth; the entrance to the larynx is surprisingly normal in most cases. The inferior vena cava can be incised just above the diaphragm, and the incision continued downward to open the intrahepatic course of the inferior vena cava, the hepatic veins, and ductus venosus. This should be done with care when an umbilical venous catheter has been passed (Fig. 2.19).

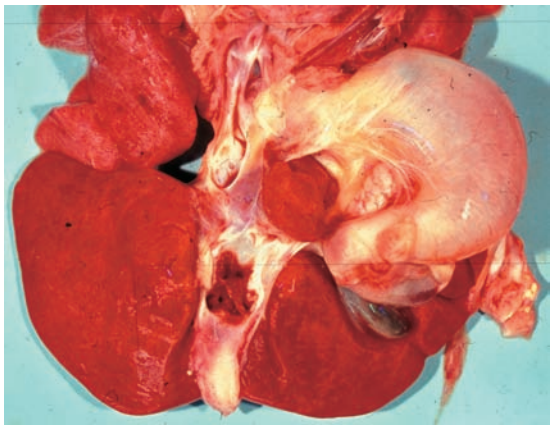

Figure 2.19. Liver from behind, inferior vena cava opened. There is thrombus emerging from the hepatic vein; post umbilical venous catheterization.

Examination of the Heart

It is now convenient to turn over the remaining viscera and continue dissection in the front. The apex of the heart is lifted up and the entry of the inferior vena cava into the heart noted. If pulmonary venous return is to the heart, the inferior vena cava is divided, and the liver removed and weighed.

It is always easier to open the heart while it is attached to the lungs from the point of view of both stability and chamber identification. It is obligatory in those cases where congenital heart disease is suspected. Definitions of cardiac structures are given in Chapter 21. The general sequence of examination of the heart described by Langley (1971) is, with minor modifications, suitable for examination of all infant hearts. Direction and sequence of incisions are displayed in Fig. 2.20. I find that it is easier to examine the heart in the fresh state than after fixation; manipulation is easier and the anatomy can be demonstrated to attendant clinicians at the same time. Details of cardiac dissection are set out in Chapter 21.

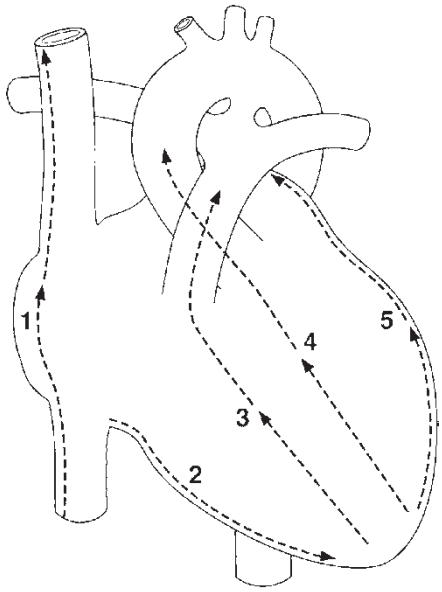

Figure 2.20. Diagrammatic representation of incision lines to display the infant heart. The heart is opened while it is attached to the lungs.

Head and Cranial Contents

Examination of the brain and its coverings in the newborn requires a different approach from that used when examining older children and adults. The scalp is incised coronally starting behind one ear, cutting posterior to the vertex toward the other ear. This incision permits satisfactory reconstruction of the head should further viewing of the body be required; it also imparts a degree of stability should skull bones be retained for examination. The scalp flaps are reflected forward and backward and examined for the presence of edema or hemorrhage (see Chapter 13). The size of the anterior fontanelle and width of suture lines are noted. Where there is increased intracranial pressure, particularly of recent origin, the fontanelle is tense and bulging (Fig. 2.21) and the suture

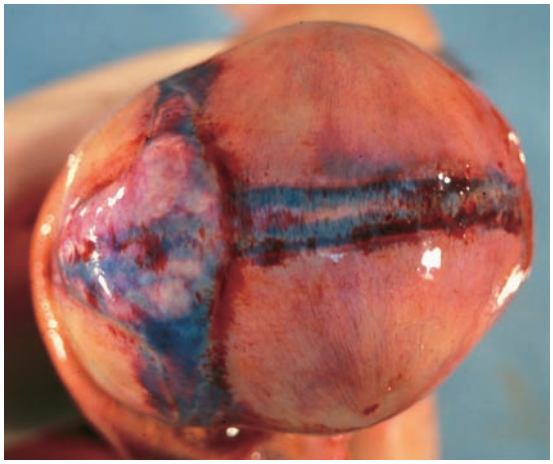

Figure 2.21. Bulging anterior fontanelle and tense, wide suture lines; posthemorrhagic hydrocephalus.

lines may be wider than normal. When there is long-standing increased intracranial pressure, the anterior fontanelle often extends forward so that the frontal bones are widely separated along their whole length. This separation is seen in some dysmorphic syndromes such as trisomy 18 as well as hydrocephalus of long standing. The appearance of skull bones and the position of any fractures or skull deformity are noted. Circular or oval areas of thinning in the bone, sometimes with a central defect (craniolacunae), may accompany long-standing hydrocephalus.

The posterior scalp flap should be reflected to the level of the cervical spines so that the whole of the occipital bone is exposed (Wigglesworth and Husemeyer 1997); any dislocation of its component parts will be apparent because of interruption of the normal contour of the bone and undue mobility between the squamous and lateral parts (see Fig. 13.25 in Chapter 13). The posterior margin of the foramen magnum is identified and the atlanto-occipital membrane incised. A sample of cerebrospinal fluid (CSF) for bacteriological culture is conveniently taken at this point with sterile pipette or needle and syringe; the CSF sample should be examined for blood staining or opacity. Herniation of the cerebellar tonsils is sometimes seen when the brain is swollen. If there is hydrocephalus, the upper cervical vertebral arches may be removed to look for malformation and downward displacement of the cerebellum and medulla oblongata, which usually accompany meningomyelocele.

The upper cervical cord is divided horizontally from the back. The cranial cavity is now opened. It is here that necropsy technique differs most from that appropriate to the examination of older children and adults because of the need to examine the supporting dural folds, which may be damaged during delivery. The anterior fontanelles are incised parasagittally with a scalpel, taking care that the sagittal sinus is not damaged. Incisions are extended forward and backward on each side in turn (Fig. 2.22). Right-handed prosectors find this part of the examination easier if they start with the left side, as they are able to support the opened side of the head more efficiently when examining the second side. The scissor point should be kept up against the bone to avoid injury to the brain. This is often difficult when brain swelling reduces the subdural space. The fronto-parietal and parieto-occipital suture lines are incised on each side.

The frontal and parietal bone flaps can be deflected laterally and the surface of the brain inspected on each side in turn. The presence, site, and size of any hemorrhage is noted, together with the appearance of the surface of the dura and arachnoid membranes. The size of the subdural

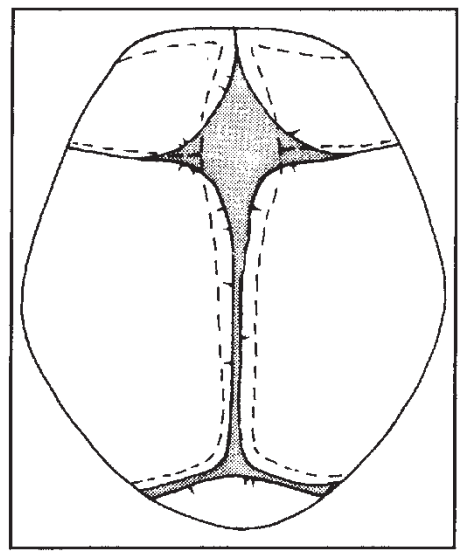

Figure 2.22. Cutting lines used to expose the brain without injury to the dural folds or venous sinuses. (Courtesy of Dr. S.A.S. Knowles, Exeter.)

space is noted; this is relatively large in the preterm baby and can be obliterated by cerebral edema or hydrocephalus. When the dura and leptomeninges have been in contact for a few days, their shiny surfaces are lost and instead appear roughened.

The cerebral gyral pattern is observed. It is a useful marker of maturity as cerebral development is usually maintained despite the presence of growth restriction of sufficient severity to affect body measurements and even head circumference. The appearance of the normal gyral pattern is surprisingly uniform, and its increasing complexity with fetal maturity is well documented by Dorovini-Zis and Dolman (1977; Fig. 2.23). Observation of cerebral gyral pattern in macerated stillbirths (see Chapter 10) may assist the dating of fetal death, even when it has occurred some weeks before delivery. The cerebral gyral pattern may be abnormal when there is major cerebral malformation and largely obliterated by severe hydrocephalus; such abnormal appearances alert the prosector to these possibilities.

The head is then tipped forward and laterally and the occipital pole gently lifted with a finger or

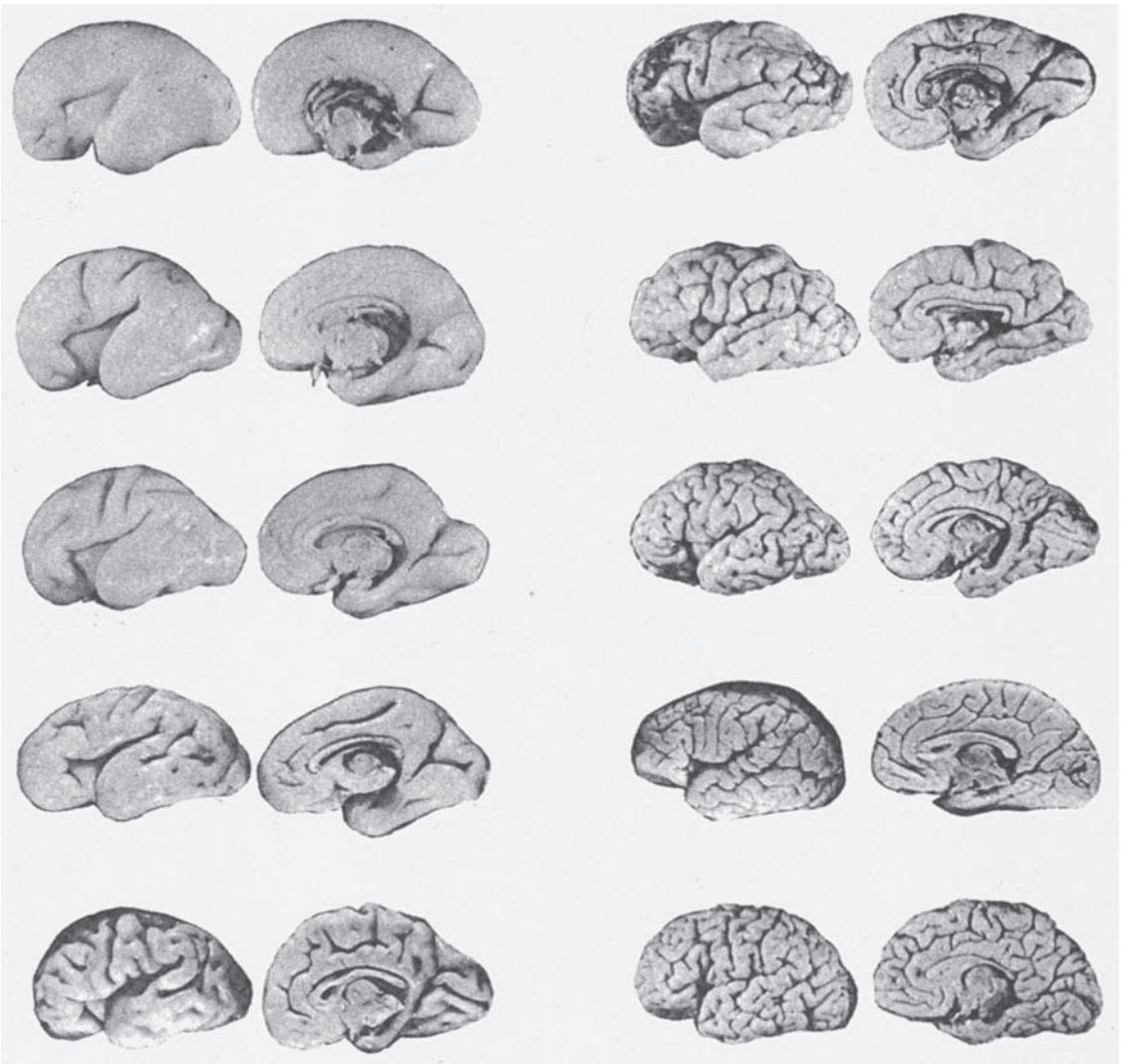

Figure 2.23. Gyral pattern of the fetal brain from 22 to 40 weeks' gestation (brains brought to same size) (Dorovini-Zis and Dolman 1977).

scalpel handle so that the falx and tentorium can be inspected for hemorrhage and tears. Congestion or focal hemorrhage within the dural folds is often seen and is nonspecific. Tearing of the supporting membranes of the brain may occur when excessive deformity of the head has occurred during rapid spontaneous or instrumental delivery. It is possible to examine the whole of the falx and tentorium in this way. The usual site of traumatic injury is the junction of the two folds (see Fig. 13.24 in Chapter 13); it may be partial, when tearing of the surface fibers is seen, or complete, with disruption of the concave margin of the tentorium. The vein of Galen and its tributaries are seen at the confluence, when they are intact. It is not necessary to remove the cerebral hemispheres to make this examination unless photography is contemplated. Following this maneuver, the skull flaps are returned to their original position and kept in place with a hand while the procedure is repeated on the other side.

The brain is now removed. The sagittal sinus is divided anteriorly and lifted up and backward. It should be inspected for the presence of antemortem thrombus, which can be secondary to severe brain swelling. The head is tipped backward so that the frontal poles can be lifted out of the anterior cranial fossa. The nerves and vessels are divided in turn and the tentorium is incised in the same manner as that adopted for removal of the adult brain. Because of the soft consistency of the infant brain as the result of incomplete myelination, especially marked in preterm babies, removal of the brain may be facilitated if the body is supported by an assistant and the head is partly immersed in a wide container of fixative so that the brain is supported on all sides as removal proceeds. The base of the brain can be inspected through this supporting fluid and the need for suspension of the brain to maintain its normal contour during fixation is removed by using a hypertonic solution of fixative.

Isaacson (1984) describes a similar method for removal of infant brains under water. He also suggests injecting gelatin mixture into the ventricles after fixation and immersing the whole brain in gelatin solution. This is allowed to set in refrigeration overnight and then sliced with a hot knife. This method would certainly support the brain well, although ventricular injection should be undertaken with care so that pathological abnormalities are not disturbed. Wigglesworth (1984) describes removal of the brain of macerated stillbirths within the protection of the dural membrane, which is removed only after fixation.

The base of the skull is inspected following removal of the brain. Dural sinuses are incised and the pituitary inspected and removed by incising the posterior clinoid processes and dissecting free the gland anteriorly, while grasping a posterior clinoid process to prevent crushing of the gland.

The middle ears may be opened with bone forceps to permit bacteriological samples to be taken or the squamous bone removed intact (Kelehan 1984). The middle and inner ears are then examined after fixation.

Much useful information is lost if the infant brain is cut in the fresh state, although the yellow staining of kernicterus will be eluted by prolonged formalin fixation. The soft consistency permits collapse, and the extent of ischemic injury is difficult to evaluate. Any blood in the ventricles spills out and its amount may go unnoticed when the prosector is distracted by disintegration of the brain. It is not possible to slice the fresh brain thinly, and focal pathological abnormalities may go unobserved.

After fixation, the brain is weighed and a second inspection of the brain surface is made; the cerebellum and brainstem are detached by dividing the cerebral peduncles and weighed. The cerebrum may be sliced coronally in the traditional manner, the first slice being made from the basal aspect at the level of the mamillary bodies. A method of blocking the neonatal brain for histological examination is illustrated in Fig. 2.24. A device to facilitate brain cutting to produce slices in the planes of any premortem computed tomographic (CT) scan is described by Muller and McCombs (1984). This could also be used to produce slices that correspond with the progressive obliquity of plane of ultrasound scans performed through the anterior fontanelle.

A technique for the removal of the brain and cord in continuity, protected by skull bones and spinal column, is described by Laurence and Martin (1959). This method is particularly useful for removal of the brain when there is marked hydrocephalus from any cause (Fig. 2.25), particularly when myelination is incomplete. The

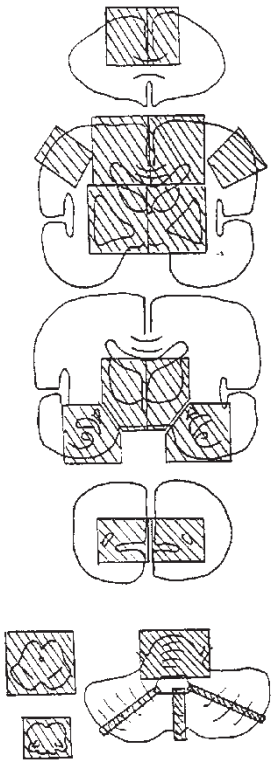

Figure 2.24. Standard blocks of fetal brain >30 weeks' gestation; total 20 blocks plus blocks of any lesion. (Dr. J.E. Bell, Edinburgh.)

authors suggest that the best results are achieved when prior fixation of the brain by perfusion of the carotid arteries with 10% formol saline is undertaken. This is generally unacceptable because of its effect on the face. I have found that replacement of some of the CSF with formalin by needling the anterior fontanelle several hours before necropsy is a satisfactory alternative. Although the procedure sounds like a formidable undertaking, it is not difficult, and the dissection is very clearly described. A modification I use is to leave the superior orbital margin intact by sawing through the frontal bones and the orbital roof. The brain does not prolapse through this small space, and the technique facilitates circulation of fixative in the subdural space (Fig. 2.26). Dissection is continued after fixation. In the absence of appropriately skilled assistance to ensure the accuracy of a parasagittal saw cut to expose brain and cord, I find that nibbling bone away with bone forceps and cutting with heavy scissors is satisfactory. Particular attention should

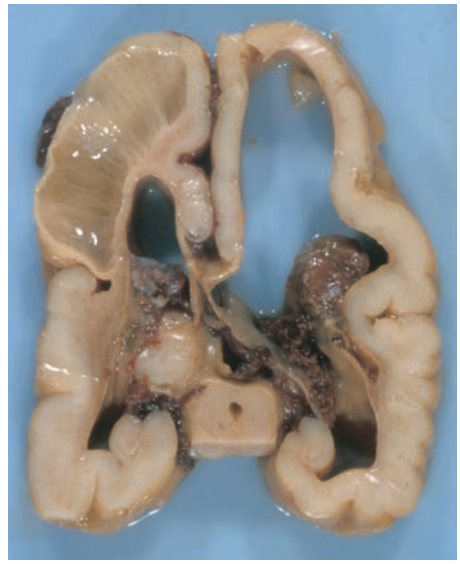

Figure 2.25. Brain slice from an infant born at 28 weeks' gestation, death at 58 days. Removal of brain within the skull ensured minimal distortion during fixation despite hydrocephaly, hemorrhage, and cystic leukomalacia.

be paid to the CSF pathway from the third ventricle to the upper cervical region of the spinal cord. It may be appropriate to block all slices of tissue through the aqueduct region if it cannot be followed easily.

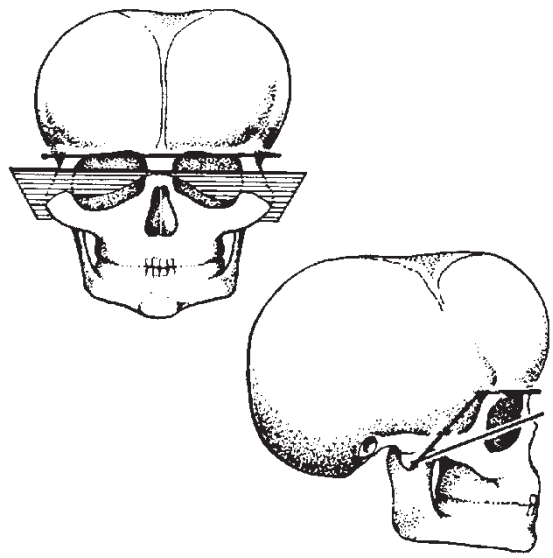

Figure 2.26. Plane of saw-cut to detach the calvarium form the facial skeleton. The upper plane is the one I use, the lower is that described by Laurence and Martin (1959).

Should one be presented with a stillbirth where hydrocephalus was recognized prenatally and decompression of the head undertaken to facilitate vaginal delivery, the above technique is inappropriate. The cranium can be opened in the usual way. The hemispheres will be collapsed but not extensively disrupted and there is usually much hemorrhage. The inferior parts of the cerebral cortex, midbrain, cerebellum, and brainstem are usually undamaged. They can be removed in continuity and fixed in formalin, together with part of the cortical mantle. After fixation the appearance of the cortex is noted, looking for micropolygyria and ependymal granularity or cortical necrosis as evidence of infection and for brown staining or organizing thrombus as evidence of past hemorrhage. The rest of the brain is sliced vertically in 2- to 3-mm slices, the CSF pathway is traced, and samples taken for histological examination.

Spinal Cord

The spinal cord in the neonate is most easily approached from the front. An intervertebral disk in the lower lumbar region is incised, and the pedicles are divided on each side with heavy scissors. When two or three pedicles have been divided, the vertebral bodies may be elevated to facilitate further dissection. The pedicles are divided on each side in turn, up to the high cervical region. The filum terminale is divided and elevated with forceps and the spinal nerve roots divided in turn upward on either side with a sharp scalpel. As the brain has been removed there is no tethering at the level of the atlas.

When traumatic injury to the cervical cord is suspected, it is better to remove the cervical part of the cord within its bony coverings as described by Yates (1959). The intervertebral disk at C8-T1 is divided, and the muscle attachments on both sides of the spinal column are divided with scissors and dissection continued around to the back, keeping close to the vertebral arches. The atlas is separated from the skull from the posterior aspect. After fixation and brief decalcification, the whole specimen can be sliced at 2- to 3-mm intervals using a sharp knife (Fig. 2.27). The surface of each block is examined with a magnifying lens. This permits evaluation not only of cord injury but also of the site of any hemorrhage and injury to joints or vertebral arteries. Yates (1959) described arterial dissection and thrombosis as a result of birth injury.

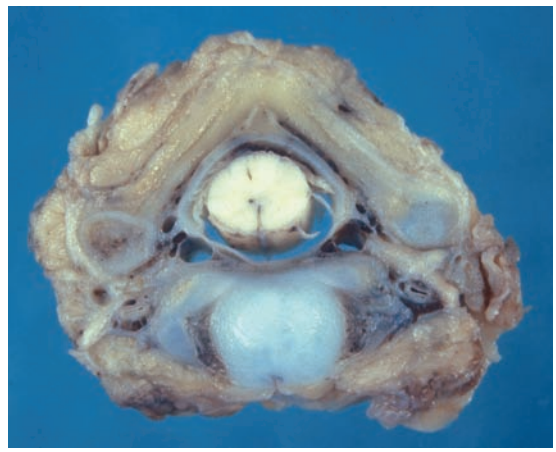

Figure 2.27. Slice through the cervical vertebral block after fixation and decalcification. Appropriate slices for histological examination can be selected with a hand lens.

Skeleton

Radiography is a more efficient technique than dissection for the recognition and documentation of skeletal malformation or injury. However, dissection of parts of the skeleton may be required in order to examine a particular joint, e.g. following recognition of congenital dislocation of the hip, or, more usually, to obtain samples of long bones for histological or biochemical examination.

The femur is removed in a similar manner to that used in adults. The ventral abdominal incision is extended through one groin, down the anteromedial border of the thigh, to the medial aspect of the knee. The anterior thigh muscles are divided vertically down to the bone, and the knee ligaments divided. The lower end of the femur is elevated, and posterior muscle attachments divided progressively in a cephalad direction. Muscles around the hip joint are divided. The joint capsule is incised. The ligament of the femoral head is exposed by elevation and lateral rotation of the femur and incision of the joint

capsule inferiorly; the remaining ligaments are then divided.

In babies with osteochondrodystrophies or osteogenesis imperfecta (see Chapter 29) it may be desirable to remove several long bones. In the interests of reconstruction and aesthetics, the following method for removal of bones is preferred. The proximal limb joint (hip or shoulder) is approached by lateral reflection of the skin from the ventral incision and division of muscles vertically downward onto the joint, which is then disarticulated. The proximal end of the bone is grasped and muscle attachments divided progressively in a peripheral direction, invaginating the "sleeve" of muscle, subcutaneous fat and skin. The next joint may be left intact and dissection continued to the ankle or wrist, which is then disarticulated. The bones are then fixed in formalin and the limb reconstructed by filling the bone space with wadding. Radiographic examination of individual bones is desirable (Fig. 2.28) before histological examination is undertaken. The bones may be usefully divided longitudinally so that undecalcified and conventional sections of the same bone can be compared.

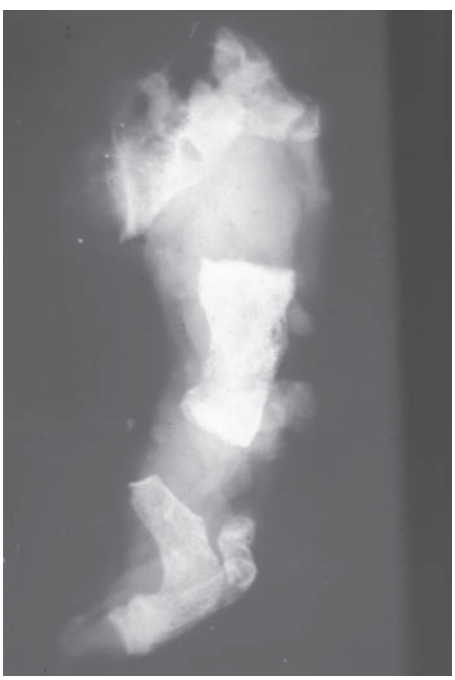

Figure 2.28. Radiograph of leg and arm following fixation, from a neonate with osteogenesis imperfecta, shows fine detail of bone pathology.

Histological Examination

Routine sampling of fetal organs for histological examination has been a most useful part of the investigation of perinatal death, producing essential information about the likely cause or mode of death in 20% of both stillbirths and neonatal deaths (Porter and Keeling 1987). Naeye (1983) analyzed his department's experience of the value of routine histology in normally formed perinatal deaths and found positive information gained in many cases. He also stressed the need to look beyond the cause of death when undertaking postmortem examination of babies; many positive findings in his analysis had clear indications for unit audit and patient management. Pryse-Davies (1981) found routine histological examination to be useful for confirmation of necropsy diagnoses in 80% of his cases, where a diagnosis was reached after gross examination of fetus and placenta, and essential in the remaining 20%.

The usefulness of histological examination in macerated stillbirths is often questioned, even by those who recognize routine histology as an essential part of perinatal necropsy (Langley 1971; Pryse-Davies 1981). Examination of major organs and the use of trichrome stains often yields useful information. In some cases, it reveals specific diagnoses, such as viral infection, which would otherwise have been missed (Rushton 1994).

Routine samples for histological examination at perinatal necropsy are listed in Table 2.5. Examination of the costochondral junction provides evidence of regularity of pre- or postnatal growth (Emery and Kalpaktsoglou 1967) and diagnostic information in osteochondrodysplasias (see Chapter 29). Blocking of the brain (Fig. 2.29) may be accomplished in fewer blocks in the very small preterm baby. These blocks comprise the basic examination and encompass common conditions. Additional blocks are taken from macroscopically abnormal areas.

Interpretation of histological appearances in the fetus and neonate requires an understanding

Table 2.5. Samples for routine histological examination at perinatal necropsy

All perinatal deaths	Additional blocks in neonatal deaths
Both lungs	Larynx (vertical through vocal cords)
Thymus	Trachea
Ventricular myocardium	Pituitary
Both lobes of liver	Thyroid
Kidney	Intestine
Pancreas	Gonads
Adrenals	Brain
Diaphragm	
Costochondral junction	
Cerebral cortex	
Placenta × 2	
Extraplacental membranes	
Umbilical cord	

of the normal appearance at the particular stage of development. Changes in appearance that are developmentally related occur to a different degree and at different gestational ages in individual organs. Some are sufficiently gestation-specific, for example, the kidney, to provide further evidence of gestational age. In other organs, for example, the lung, the stage of development to some extent determines the pathological abnormality found. The histological appearance of the placenta at different gestations is illustrated in Chapter 3. A reference collection of histological sections of normal organs from babies of known gestational ages is very useful for purposes of comparison.

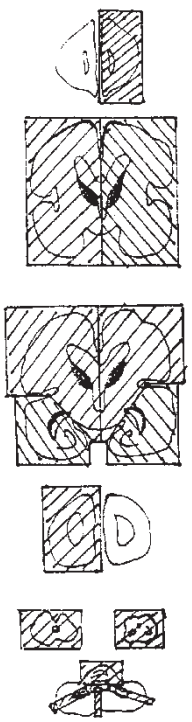

Figure 2.29. Standard blocks of fetal brain 24 to 30 weeks' gestation, total 14 blocks plus blocks of any lesion. (Courtesy of Dr. J.E. Bell, Edinburgh.)

Microbiological Examination

Pryse-Davies and Hurley (1979) examined the usefulness of cisternal CSF, heart swab, and bronchial swab as a routine screening procedure in 835 perinatal necropsies. Positive cultures were obtained from 12.1%, 12.1%, and 41.3% of samples, respectively. The authors did not find that postmortem delay increased the proportion of positive cultures and found a significant correlation between positive bronchial cultures and histological evidence of pneumonia.

The role of routine microbiological sampling during perinatal necropsy was questioned by Mueller et al. (1983) and found by them to be a cost-effective investigation. They obtained positive cultures in 4% of stillbirths and 15% of neonatal deaths. Barson (1990) found microbiological evidence of infection in only 1.8% of stillbirths, but 28% of neonates had pneumonia and 13% were septicemic.

Naeye (1983) cited recovery of organisms from 72% of placentas with histological evidence of chorioamnionitis among preterm deliveries. Among our cases (Zaaijman et al. 1982), positive culture was obtained less frequently from placentas from both term and preterm deliveries, and correlation between histological evidence of inflammation and bacteriological isolation was poor. Langley (1971) advocates samples of lung and blood from a large subchorial placental vessel for routine microbiological examination.

Many neonatal units are selective in their requests for virological investigations postmortem. This policy should extend to bacteriological examination as well (Billson et al. 1993).

Cytogenetics

Optimally, the samples should be taken from all fetal and neonatal deaths for chromosome examination. Few departments are prepared to undertake this amount of routine work and some selection of cases is necessary. The best return from chromosome examination is among malformed fetuses and perinatal deaths. Normally formed macerated stillbirths provide the next highest rate of abnormalities, followed by samples from normally formed fresh stillbirths and neonatal deaths (Alberman and Creasy 1977). Sutherland and Carter (1983) report a very low rate of chromosome abnormality in isolated anencephaly or spina bifida.

Pericardium or gonad samples are suitable from fresh fetuses. In macerated fetus, placental amnion may be more rewarding than fetal tissue and less prone to contamination by maternal cells than placental parenchyma. Common chromosome abnormalities can be confirmed or refuted using molecular techniques.

Molecular Genetics

Continued development of techniques means that formalin-fixed tissue, even after paraffin wax embedding, is suitable for many investigations. However, when facilities permit, the retention of a small, unfixed frozen sample from all malformed fetuses is recommended. Retention of samples is even more important when multiple anomalies are present.

Sampling for Biochemical Investigations

When samples of body fluids or tissues are needed for the investigation of suspected inborn errors of metabolism, they should be obtained as soon as possible after death. When there is clinical suspicion of such abnormality (see Chapter 7), which has not been confirmed or excluded during life, then colleagues should be advised to seek consent for necropsy, or, failing that, for removal of specific tissue samples before the baby dies so that delay in obtaining important diagnostic material is minimized. Delay reduces the reliability of investigations. Samples required will depend on the type of disorder suspected, but plasma, serum and urine together with skin for fibroblast culture are useful minima. Prior discussion with colleagues in the biochemistry and genetics departments may avert disappointment and unnecessary effort. Appropriate sampling is set out in Table 7.4 in Chapter 7.

Examination of the Placenta

It is important that the placentas of fetuses, stillbirths, and neonatal deaths be examined as part of necropsy examination. Additionally, placentas from some complicated pregnancies are worth examining, whatever the outcome of pregnancy in terms of fetal viability and well-being. It is not difficult to ensure that the placentas of fetuses and stillbirths are available for examination as they usually accompany the baby to the pathology department. Difficulties often arise in ensuring the availability of a placenta in the event of neonatal death. Few pathology departments are willing, or even able, to undertake the examination of all placentas delivered in their obstetric unit, even though this is the only way to ensure that useful information is not lost (Rushton 1982). Most of us find it necessary to invoke some degree of selection. One method is to hold all placentas from live births in refrigeration in individually labeled bags for 1 week. This method may have been useful when virtually all neonatal deaths occurred during the first 7 days; however, the proportion of late neonatal deaths has increased as a result of neonatal intensive care, so this method is no longer appropriate.

All placentas from babies who go to the intensive care nursery are sent to our pathology department, and all are examined, irrespective of outcome. We also examine all placentas from prematures, growth-restricted infants, and multiple pregnancies. Those from pregnancies complicated by maternal hypertension, pregnancy-associated hypertension, elevated α-fetoprotein

level in the second trimester in the absence of fetal malformation, rhesus incompatibility, and maternal immunological disorders are also examined. Any placentas thought by obstetric staff to be unusual are sent for pathological evaluation. In this way those placentas that are likely to have pathological abnormality are selected for examination, and it is probable that most neonatal deaths will have had one of these adverse factors during pregnancy. Only placentas from a few externally normal, mature babies, who are well at birth are likely to evade examination when this selection procedure is used. In practice, 15% of delivered placentas are examined in the pathology department.

Macroscopic Examination

Placentas of infants at high risk of infection (e.g., cases of prematurity, maternal pyrexia, or prolonged rupture of membranes) may have amniotic and chorionic smears and swabs taken, as described by Blanc (1981), as soon as possible after delivery. This procedure avoids problems of contamination, and examination of Gram-stained smears provides a quick diagnosis of bacterial infection. Should there be a history of maternal viral infection, if the baby is unexpectedly growth restricted or the placenta is small and pale, a block of placental parenchyma is obtained for viral studies (see Chapter 3).

Once samples have been taken, the placenta may be examined in a fresh state or fixed flat in formalin for a few days. Some lesions are easier to see in the fixed organ, while a recent infarct is easier to detect in the fresh state.

Weight and Measurement

The gross weight of the placenta is not a good indication of its functional mass. Trimmed weight (membranes trimmed to the disk margin and cord cut within 2 cm of its insertion) is a better guide. While accepting the limitations of placental weight in respect to placental function, comparison with the weight of the baby does serve to draw attention to excessively heavy or light placentas, which warrant further examination (Table 2.6). Weight standards for twin placentas can be found in Pinar et al. (1996). Two placental diameters are measured at right angles and maximum thickness is recorded.

Table 2.6. Mean weights and percentiles for singleton placentas (Pinar et al. 1996)

Gestational age (wk)	90th percentile	75th percentile	Mean singleton placental wt	25th percentile	10th percentile	Number of cases
21	172	158	143	128	114	3
22	191	175	157	138	122	6
23	211	193	172	151	133	7
24	233	212	189	166	145	9
25	256	233	208	182	159	19
26	280	255	227	200	175	14
27	305	278	248	219	192	9
28	331	302	270	238	210	16
29	357	327	293	259	229	11
30	384	352	316	281	249	12
31	411	377	340	303	269	14
32	438	403	364	325	290	24
33	464	428	387	347	311	30
34	491	453	411	369	331	32
35	516	477	434	391	352	44
36	542	501	457	412	372	36
37	566	524	478	432	391	32
38	589	547	499	452	409	62
39	611	567	519	470	426	103
40	632	587	537	487	442	193
41	651	605	553	502	456	87

Membranes

The completeness of the membranes and point of rupture are noted. Membranes are often incomplete and ragged when rupture is prolonged. The distance from point of rupture to the disk margin may indicate placenta praevia.

The color and fetal surface of the membranes are examined. Membranes are cloudy or opaque and greenish when there is bacterial infection, greenish following the passage of meconium, and brownish in cases of old retroplacental hemorrhage. Surface irregularity may be observed when there is amnion nodosum, squamous metaplasia, or certain infections such as *Candida albicans* or *Listeria monocytogenes*.

Umbilical Cord

Naeye (1985) provides gestation-related normal values of cord length (Table 2.7). Excessively long (greater than 90 cm) or short (less than 40 cm) cords are significant findings. The former predispose to entanglement, the latter may interfere with fetal descent through the birth canal.

Edema of the cord and focal abnormalities such as bruises or strictures, hematomas, hemorrhage as well as knots and varicosity, the number of cord vessels, cross-sectional area, and the site of insertion are recorded. Where there is velamentous insertion, the distance from insertion to the disk margin and the length of the membranous course of the vessels is measured. Disruption of these vessels should be carefully sought.

TABLE 2.7. Umbilical cord length at various gestational ages from 35,779 neonates (Naeye 1985)

Gestational age (weeks)	*n*	Umbilical cord length (cm)
20–21	16	32.4 ± 8.6
22–23	27	36.4 ± 9.0
24–25	38	40.1 ± 10.1
26–27	59	42.5 ± 11.3
28–29	80	45.0 ± 9.7
30–31	113	47.6 ± 11.3
32–33	337	50.2 ± 12.1
34–35	857	52.5 ± 11.2
36–37	3,153	55.6 ± 12.6
38–39	10,083	57.4 ± 12.6
40–41	13,841	59.6 ± 12.6
42–43	4,797	60.3 ± 12.7
44–45	1,450	60.4 ± 12.7
46–47	492	60.5 ± 13.0

Data represent mean ± 1 SD.
n = number of cases.

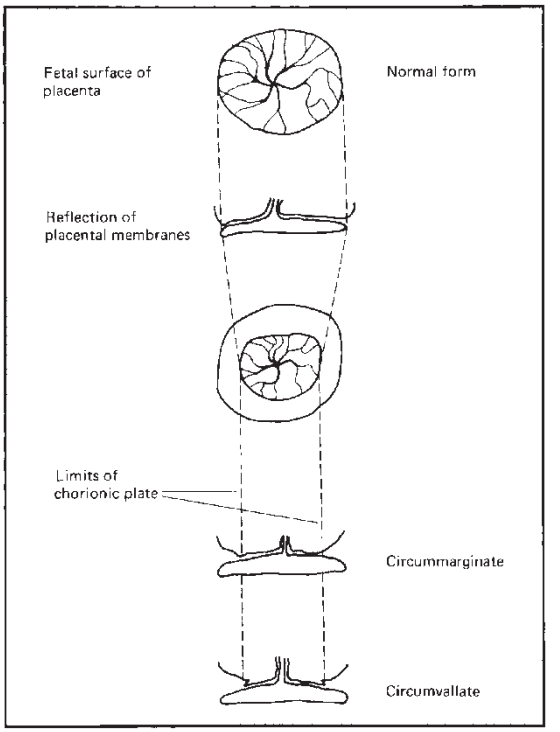

FIGURE 2.30. Different types of placentation.

The umbilical coiling index, the number of coils/cord length in centimeters, is noted. In uncomplicated pregnancies, a mean of 0.17 cm [0.009 standard deviation (SD)] was derived (van Dijk et al. 2002). Both hyper- and hypo-coiling are associated with adverse pregnancy outcome (Machin et al. 2000; de Laat et al. 2007).

Placental Surface

The fetal surface of the placenta is inspected. The course of large vessels is observed (arteries can be identified as they cross superficial to veins). Large vessels should extend to the disk margin (normal form) but stop short of it when the placenta is circummarginate or circumvallate in form (Fig. 2.30). Subchorial hemorrhage or fibrin deposition may be apparent but are of little significance.

Occasionally vascular thrombi or aneurysms are apparent.

The maternal surface is examined for completeness, adherent clot, and surface depression typically caused by retroplacental hemorrhage. Focal calcification is often apparent in the maternal surface. It is of no significance in respect to placental function.

Slicing the Placenta

The placenta is sliced (easiest with maternal surface upward) at approximately 1-cm intervals and the slices laid out in order for close inspection. Any lesions such as subchorionic fibrin, perivillous fibrin, infarcts, or cysts should be noted. Many macroscopic abnormalities can be identified during this examination, but where there is any doubt blocks should be taken for histological confirmation. Blocks should be taken from apparently normal areas of the placenta (Fig. 2.31). Obtaining representative samples from placentas is a problem, for there are quantitative structural differences between the edge and the center of the placenta and between basal and subchorionic areas (Boyd et al. 1980). For routine histological analysis, it is appropriate to take two full-thickness blocks from the placenta and a block from the margin, which is more likely to have deciduas attached. If villitis is suspected, then at least four blocks should be taken from different areas. A roll of membranes from the site of rupture and a block of umbilical cord complete the placental sampling for histological examination.

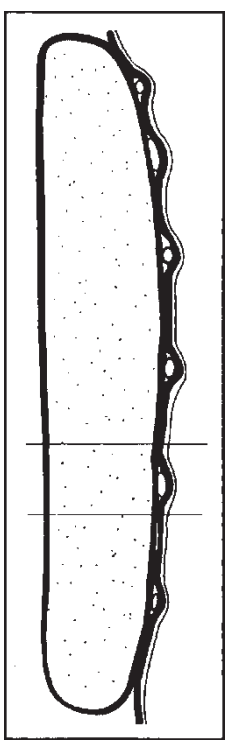

Figure 2.31. Blocks of placenta for histological examination should include both fetal and maternal surfaces.

The Postmortem Report

All of the activity discussed in this chapter is of limited usefulness if it is not communicated to clinicians in a clear and timely fashion. It may be difficult to complete all necessary investigations and issue a report within 1 or 2 weeks. A useful compromise is to send a brief note to the clinician summarizing the gross necropsy findings on the day of the examination and to ensure that a more detailed description with histological findings, radiology, and bacteriology, preferably with gross inspection of the fixed brain, will be available in time for the maternal postnatal outpatient appointment and perinatal mortality meeting. Further details such a brain histology and cytogenetic studies can be added later.

I do not think there is a place for structured report forms in perinatal pathology. The amount of description in any particular system varies enormously from case to case, so structured forms are inappropriately constricting. It is helpful to set out one's reports in the same way so that clinicians find their way around easily. The report should include all weights and measurements with normal values or a comment about normality. An opinion about fetal maturity and appropriateness of size is important. Specific questions should be answered and the clinician's attention drawn to any lesion thought to result from treatment. All pathological diagnoses should be set out at the end of the report.

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3
The Placenta

T. Yee Khong

The placenta and extraplacental membranes are an apposition of fetal and maternal tissues for the purposes of physiological exchange. The placenta has a finite life span and is the body's largest biopsy, and yet its examination is often neglected, even in cases of fetal and neonatal deaths, sick and premature neonates, and maternal complications of pregnancy. Several monographs and reviews on the placenta have provided a better understanding of its examination and pathology (Benirschke and Kaufmann 2000; Vogler et al. 2000; Khong 2001; Hargitai et al. 2004; Kraus et al. 2004; Faye-Peterson et al. 2005).

The introduction of noninvasive techniques to investigate the fetal well-being early in pregnancy, including antenatal diagnosis, has made it important for the pathologist to become familiar with the development and structure of the placenta through all stages of pregnancy. Significant pathological processes can affect the placenta before involving the fetus. Many placental and implantation site lesions can now be visualized by ultrasound, in utero magnetic resonance imaging, or Doppler ultrasound assessment of fetal, umbilical, and maternal vessels. Correlation of these findings with placental histopathology is important for quality assurance purposes. In recent times, examination of the placenta has come to assume an important role in obstetric litigation, commonly when there is perinatal death, fetal distress, or alleged cerebral hypoxia (Kraus 2003).

Placental inflammation is covered in Chapter 4 and the placenta in twins and multiple pregnancy is covered in Chapter 12.

Development of the Placenta

Embryology of Early Development

Fertilization of the ovum usually occurs in the ampullary region of the fallopian tube and results in the restoration of the diploid number of chromosomes. Cleavage begins at once, and by 3 days after fertilization a cluster of 12 cells, called a morula, is formed. This is the state in which the fertilized ovum enters the uterine cavity, the endometrium being in a luteal phase. Fluid then accumulates within the morula, and trophoblast differentiation results in the formation of the blastocyst, which begins to attach to the uterine mucosa about 6 days following fertilization.

During the second week the outer layer of the blastocyst proliferates to form the trophoblastic or chorionic shell, while the inner layer differentiates into a bilaminar embryonic disk, which then separates from the trophoblastic layer by the formation of a cavity that ultimately becomes the amniotic space. The primitive trophoblast differentiates into an inner layer composed of mononuclear cells known as the cytotrophoblast and an outer layer consisting of multinucleated cells known as the syncytiotrophoblast. Intercommunicating fluid-filled spaces appear in the rapidly enlarging trophoblastic mass from the 8th day. These coalesce to form a lacunar system that opens into maternal sinusoids, presumably derived from endometrial capillaries. With the involvement of more capillaries and, later, venous sinusoids the lacunae fill with maternal blood and endometrial

glandular secretions to form a primitive intervillous space.

Invasion of the endometrium, which began on the 7th day after fertilization, is completed by the 12th day. The initial site of implantation is covered by a plug of blood clot and cellular debris, but by the 12th day the endometrial epithelium is reconstituted, resulting in an interstitial implantation.

The endometrial stromal cells around the conceptus enlarge and accumulate glycogen and lipid. The vascular and glandular changes in the endometrium together with the stromal cellular changes are known as the decidual reaction, which soon spreads to involve the entire endometrium. Until the 4th month of gestation, three regions of the decidua are identified according to their relation to the implantation site. The decidua basalis is the part underlying the conceptus, while the decidua capsularis is the superficial portion of the decidua overlying the conceptus. The remainder of the uterine cavity is lined by the decidua parietalis or decidua vera, which ultimately fuses with the decidua capsularis at about the 4th month of gestation, thus obliterating the uterine cavity (Fig. 3.1).

The last 2 days of the 2nd week are characterized by the appearance of chorionic villi. The lacunae are incompletely separated from each other by columns of syncytiotrophoblast. Although the system is labyrinthine rather than villous and none of the trabeculae have free ends, nevertheless they are called primary villous stems. They possess a central core of cells derived from proliferation of cytotrophoblast at the chorionic base. With continuing development and expansion of the implantation site, the primary villous stems increase in length and their cytotrophoblastic cells extend distally toward the attachment of the syncytium to the endometrium.

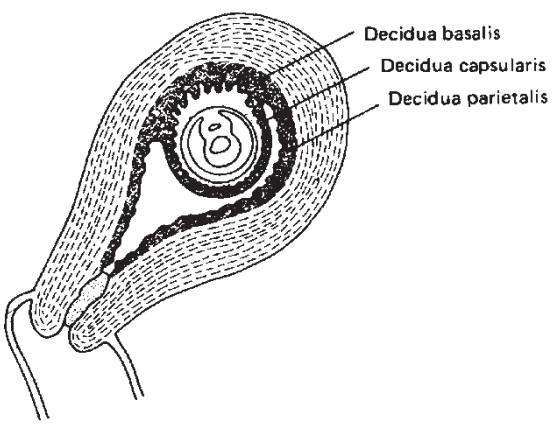

FIGURE 3.1. Development of the decidua in early pregnancy.

During the 3rd week after fertilization the bilaminar disk is converted into a trilaminar embryo composed of the three definitive germ cell layers. It also includes the development of the primitive streak, notochord, and somites. The primary villi develop mesenchymal cores, converting them into secondary villi. These branch and cover the entire surface of the chorionic shell. Differentiation of the mesenchymal cells into vascular capillaries converts the villi into tertiary villi. These villous vessels soon become connected with the embryonic heart via vessels that differentiate in the mesenchyme of the chorion and connecting stalk. By the end of the third week, completion of the development of arteriocapillary–venous network within the villi and contraction of the embryonic heart result in a true embryonic and villous circulation. Nutrients are absorbed from maternal blood in the intervillous space and products from the fetus are excreted back across the villi into the intervillous space.

Concurrent with the development of the secondary and tertiary villi, cytotrophoblastic cells derived from the tips of the villi break through the syncytiotrophoblastic layer and expand laterally to meet and fuse with adjacent columns to form a cytotrophoblastic shell that completely surrounds the conceptus. Thus, the primitive syncytium is split into an inner layer, the definitive syncytium, and an outer layer separating the cytotrophoblastic shell from the decidua. The outer layer eventually degenerates, being replaced by fibrinoid material known as Nitabuch's layer.

Development of the Definitive Form

During the 4th to 8th week the embryo develops limbs and all internal organs are formed. By the end of the 16th week the external genitalia are well defined and ossification has commenced. During this period the placenta attains its definitive form and thereafter undergoes no further major modifications.

Establishment of the cytotrophoblastic shell results in a rapid circumferential extension of the implantation site. The intervillous space expands and the primary stem villi now branch. Like their predecessor, these branches initially consist of syncytiotrophoblast but are rapidly invaded by cytotrophoblast, mesenchymal core, and fetal vessels.

Initially the entire chorion is villous, but differential growth results in the formation of the definitive placenta. The villi on the surface of the chorion adjacent to the decidua basalis continue to proliferate to form the chorion frondosum, which becomes the definitive placental parenchyma. The villi of the decidua capsularis regress, leaving a smooth relatively avascular area known as the chorion laeve. The regression of these villi is accompanied by the loss of associated intervillous space in this area. As the conceptus enlarges, the chorion laeve bulges into the uterine cavity to fuse with the decidua parietalis, thus obliterating the uterine cavity.

During this period there is some regression of the cytotrophoblastic elements in the cytotrophoblastic shell and chorionic plate, but remnants of cells are found in the former location to form the cytotrophoblastic cell islands in the layer of Nitabuch. Cytotrophoblast derived from these remnants in the cytotrophoblastic shell and from tips of anchoring villi continue to migrate into the decidua basalis and myometrium to play a key role in the development of the uteroplacental circulation (vide infra).

The chorion frondosum continues to grow rapidly. Placental septa first appear in the 3rd month of gestation. It is now generally accepted that the septa are formed partly as a result of differential growth of villi relating to a given stem villus with compression of decidua in the regions where villous growth is more vigorous, and partly by the pulling up of the basal plate into the intervillous space by relative diminished growth of some anchoring villi. The septa are formed of variable amounts of maternal and fetal tissue making up the basal plate and have no functional significance. These septa partially divide the placenta into 15 to 20 lobes.

By the end of the 4th month of pregnancy the placenta has attained its definitive form. Growth continues by further arborization of the stem villi and production of new villi. The proportion and size of the fetal components of the placenta increase as gestation proceeds. There is continuous growth until term, although the rate of increase gradually decreases from approximately 34 to 36 weeks' gestation; this has been confirmed by ultrasound determination of placental volume (Geirsson et al. 1985). Morphometric studies indicate that the villous surface area continues to increase until term (Boyd 1984), while the quantity of DNA as an indicator of nuclear (not cell) number in the placenta continues to rise linearly until term, thus dispelling the concept of placental senescence.

Mature Placenta

Macroscopically the placenta shows wide variation in shape, size, and weight. The fetal surface is normally shiny and bluish. The most superficial layer is the amnion, through which large vessels running to the umbilical cord insertion are seen. Arteries and veins may be distinguished as arteries lie superficially to the veins. A small white plaque is commonly seen beneath the amnion surface; this is the yolk sac remnant and is of no clinical significance, although the presence of two in a singleton placenta may alert one to the possibility of a vanishing twin syndrome. Insertion of the umbilical cord is variable. The chorionic plate lies beneath the amnion. Subchorionic fibrin deposition is variable and may obscure the subchorial lake. Blood vessels course perpendicularly through the chorionic plate toward the decidua. Beneath the chorionic plate lie chorionic villi and the intervillous space.

Calcium deposition is commonly seen near term and is usually at or near the maternal surface, within old infarcts, septa, and subchorionic fibrin plaques. Remnants of the maternal decidua may be adherent to the basal plate. The basal plate forms the floor of the placenta. The basal plate or maternal surface is loosely divided into 15 to 20 cotyledons that are demarcated by septa, which are infoldings of decidua and extravillous trophoblast pulled up into the placental parenchyma. Extraplacental membranes extend from the lateral margin of the placenta and form, in utero, a closed cavity containing the fetus and approximately 500 mL of amniotic fluid.

Villous Structure: Vasculature and Histology

Each primary stem villus gives off several secondary stem villi, each of which forms a lobule by division into tertiary stem villi, which are arranged in a circular manner running toward the basal plate and leaving a central, relatively villus-free area in the center of the lobule. The tertiary stem villi turn back from the basal plate and break up into terminal villi (Fig. 3.2).

The intermediate villus has been proposed as an additional type to the stem and terminal villi. The stem villi, responsible for the mechanical stability of the villous tree, is defined on the basis of containing blood vessels with media, and detailed studies have shown that these ramify further peripherally than originally thought. Branches arising from these stem villi are either smaller stem villi or intermediate villi. Intermediate villi are defined on the basis of a reticular stroma with vessels lacking media and are located between the stem and terminal villi. Two types of intermediate villi are distinguished: the mature intermediate villus, seen in the mature placenta, which is slender and bears a large number of terminal villi, and the immature intermediate villus, which is thicker and with fewer terminal villi arising from its surface. The immature intermediate villus corresponds to the immature villus that was thought to be not yet fully developed terminal villus. In the schema of this classification, as pregnancy progresses, the number of immature intermediate villi is reduced with only a few persisting to term, tending to be in the centers of functional placental circulatory units acting as growth centers, and the growth of the placenta slows down accordingly.

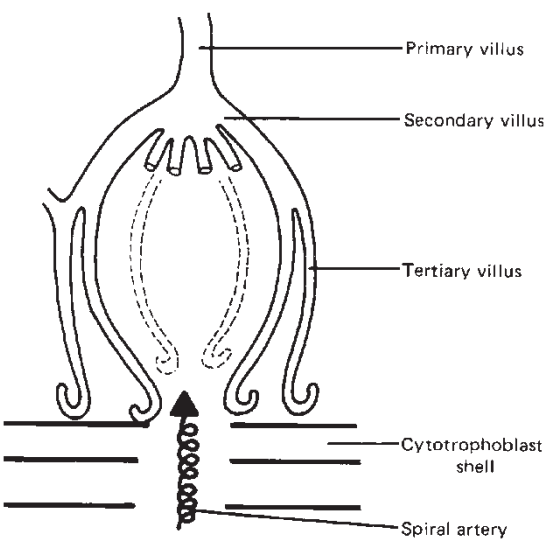

FIGURE 3.2. Structure of a placental lobule.

The terminal villi are the sites for maternofetal and fetomaternal exchanges and are the final branchings of the villous tree.

In early pregnancy the villi are relatively few and large (approximately 170 µm in diameter). There is a regular double layer of trophoblast, the outer syncytiotrophoblast and inner cytotrophoblast (Langerhans' layer) enclosing a loose stroma. At this stage fetal capillaries are small and centrally placed. The villous core also contains stellate mesenchymal and Hofbauer cells, the latter acting as fetal macrophages. As the pregnancy and the placental growth proceed, new terminal villi form by the development of trophoblastic sprouts, which are then invaded by stroma and fetal capillaries. Those sprouts that do not form villi tend to become pedunculated and break off to be deported and lodge in the maternal pulmonary vasculature (Fig. 3.3).

As pregnancy proceeds, terminal villi become more numerous and smaller (average diameter of 70 and 40 µm in second and third trimesters, respectively). The cytotrophoblast becomes less numerous and has largely disappeared by term, although its regenerative potential remains. The syncytiotrophoblastic layer becomes gradually thinner, and the fetal capillaries occupy an increasing cross-sectional area of the villus and move more peripherally to eccentric positions beneath the syncytiotrophoblast (Fig. 3.4). In some areas the fetal capillaries appear to fuse with the overlying attenuated syncytiotrophoblast to form vasculosyncytial membranes that are believed to be the optimal area for gaseous exchange (Fig. 3.5). While the vascularity of the villus increases, the villous stroma becomes increasingly condensed, and Hofbauer cells, though present at term, may be difficult to visualize because of compression by vessels and stromal tissue.

Syncytial knots formed by the sequestration of nuclei of the syncytiotrophoblast increase in number in the last 2 months of gestation. They

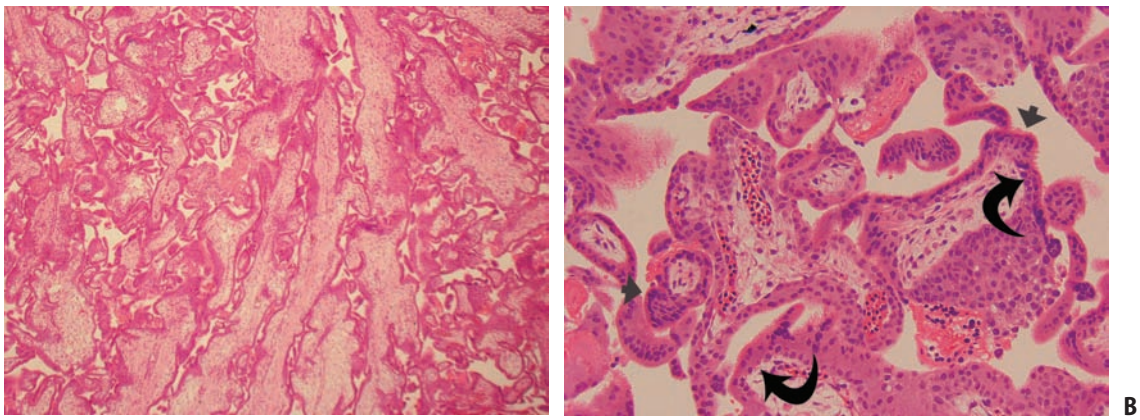

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Figure 3.3. (A) Placental villi at 8 weeks' gestation. (B) High-power view showing trophoblastic sprouts (arrowhead), prominent cytotrophoblast layer (curved arrows), and nucleated fetal red blood cells within the vessels.

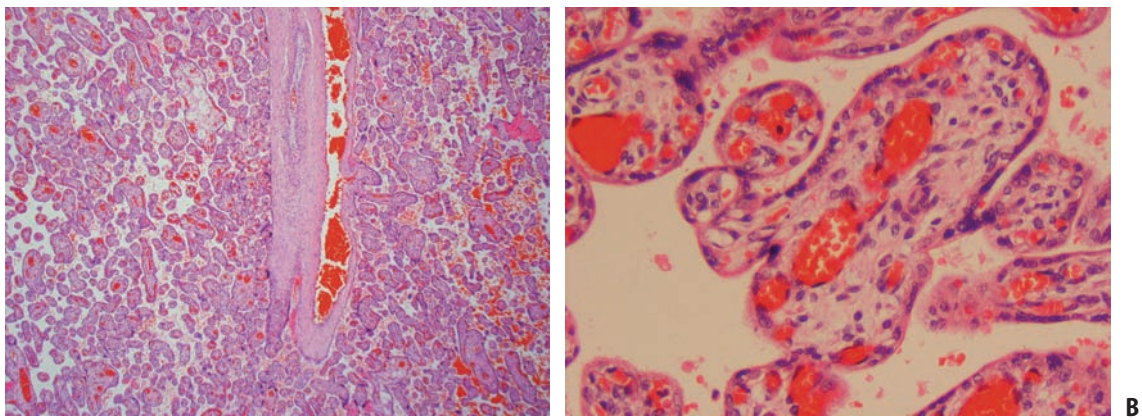

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Figure 3.4. (A) Placental villi at 27 weeks' gestation. (B) High-power view showing reduced cytotrophoblastic layer, readily evident Hofbauer cells, and increasingly eccentrically placed vessels.

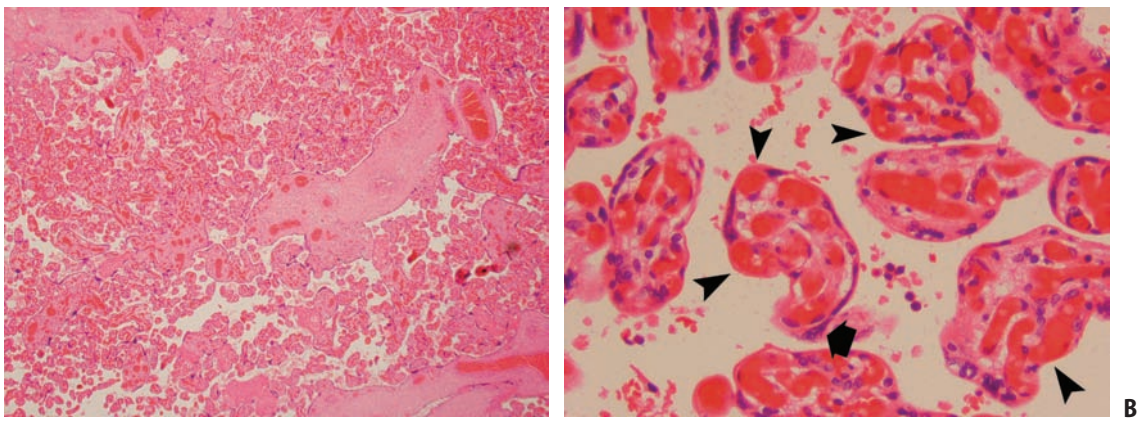

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Figure 3.5. (A) Placental villi at 38 weeks' gestation. (B) High-power view showing numerous vasculosyncytial membranes (arrowheads). Occasional syncytial knot is evident (arrow).

may bulge into the intervillous space, where they meet similar knots from adjacent villi to form an intervillous bridge. Toward the end of pregnancy fibrinoid increasingly is deposited on the surface of the villi.

Electron Microscopy

The villous cytotrophoblast is relatively simple at the ultrastructural level, with few cytoplasmic organelles. It has a large nucleus with prominent nucleolus, and the cytoplasm contains large mitochondria, a few well-developed Golgi bodies, and abundant free ribosomes. This ultrastructure is appropriate for its role in growth and differentiation. Syncytiotrophoblast is electron-dense with many organelles related to its role in steroid and protein synthesis and transport. It contains abundant dilated rough endoplasmic reticulum, pinocytotic vacuoles, free ribosomes, and numerous glycogen granules. Well-developed Golgi apparatus and mitochondria are present. The surface has a microvillous border associated with pinocytotic vesicles and vacuoles. Scanning electron microscopy has revealed large numbers of densely packed microvilli in the first trimester. As pregnancy proceeds they become less dense, blunter, and variable in size and focally absent. They are reduced in number over vasculosyncytial membranes.

Morphometry

Morphometry allows macroscopic and microscopic appearances to be quantified. While measurements on the fixed delivered placenta may not relate directly to the organ in vivo, they provide useful information for comparative analysis during ontogeny and in normal and abnormal pregnancies. The latter is of particular utility as the pathological abnormality may be quantitative rather than qualitative. Mayhew and Burton (1988) have laid out the requirements for useful and meaningful morphometric studies in placentas based on sound sampling and standardized preparative steps. They point out that the extant data on abnormal pregnancies are not comparable from center to center because of different sampling and preparative methods. The main criticism is that many investigations have used the immersion fixed placenta while they advocate perfusion fixed, and ideally dual-perfusion fixed, placenta for study.

In general, however, the villous surface area seems to be significantly lower in placentas from women with severe preeclampsia or with small-for-gestational-age infants uncomplicated by hypertensive diseases. A larger surface area was found in those with diabetes mellitus.

Development of the Membranes

By the 8th day following fertilization, a slitlike cavity destined to be the amniotic cavity, is formed superior to the bilaminar embryonic disk, whereas inferiorly a cavity that initially is the blastocyst cavity and later the primitive yolk sac cavity is formed. With formation of the extraembryonic coelom, the primitive yolk sac decreases in size and a secondary yolk sac develops. These sacs are surrounded by the developing extraembryonic coelom, extraembryonic mesoderm, and trophoblast. The embryo, amnion, and yolk sac suspended by the connecting stalk are thus contained within the chorionic sac. As the amniotic sac enlarges, it obliterates the chorionic cavity and adheres to the chorion, covers the umbilical cord and displaces the receding yolk sac to the base of the umbilical cord, this process being completed by 12 weeks' gestation.

The amnion is multilayered with a cuboidal epithelium lying on a well-defined basement membrane, deep to which are the compact, fibroblast, and spongy layers. The compact layer is relatively resistant to leukocyte infiltration and confers the strength of the amnion, while the fibroblast layer permits distensibility. The spongy layer consists of collagen fibers, mucus, fibroblasts, and macrophages. The chorion is also multilayered comprising cellular and reticular layers, pseudomembrane, and trophoblast. Although the cytotrophoblastic cells in the chorion laeve do not appear to have marked secretory activity, there is increased rough endoplasmic reticulum, suggesting continued functional capacity.

The amnion and chorion grow until approximately 28 weeks' gestation, after which mitotic activity is rare. Subsequent enlargement of the chorioamniotic sac takes place by stretching.

Development of the Umbilical Cord

At the beginning of fetal development there are two stalks: the yolk sac stalk containing the vitelline duct and vitelline vessels, and the connecting stalk containing the allantois and umbilical vessels. These two stalks fuse to form the umbilical cord. The umbilical cord is covered by amnion that is continuous with the outer layer of the embryo. The blood vessels in the allantois become the umbilical vein and arteries and are supported by Wharton's jelly. Anastomoses between the arteries often occur close to the placenta and occasionally the arteries fuse.

Development of the Uteroplacental Circulation

The early involvement of the subepithelial capillaries and, later, the arterioles and veins in the lacunar and early intervillous stages of development of the placenta has been described. Concurrent with the formation and development of the placenta, there is an invasion of maternal decidua basalis and subjacent myometrium by trophoblast proliferating from the cytotrophoblastic shell and, later, from the tips of anchoring villi. There are two types of placental bed extravillous cytotrophoblast: interstitial and endovascular trophoblast.

Interstitial trophoblast tends to be concentrated around spiral arteries at both the decidual and myometrial levels and its migration continues for the first 6 months of pregnancy, although invasion of the myometrium is probably most prolific during the first 18 weeks of gestation (Pijnenborg et al. 1981). There is a relative absence of mononuclear cytotrophoblast in the myometrium at term, suggesting that many such cells have either disappeared or the majority have been transformed into characteristic syncytial placental bed giant cells by symplasmic fusion, leading the unwary to the erroneous diagnosis of "syncytial endometritis."

Cytotrophoblastic migration into the spiral arteries appears to occur in two waves—the first wave from about 6 weeks' to about 12 weeks' gestation, and the second wave from about 16 to 22–24 weeks' gestation. Spatially the first wave affects predominantly decidual segments, while the second wave of trophoblastic migration colonizes the myometrial segments of the spiral arteries. The endovascular trophoblast penetrates the vessel wall through the endothelial lining, disrupting the intima, internal elastic lamina, and much of the muscular media, the process being accompanied by the deposition of fibrinoid material. These extensive structural alterations in the walls of the invaded arteries result in their conversion to uteroplacental arteries, and as these changes are obviously adaptations to pregnancy, the term *physiological changes* was used to describe them. While the veins draining the intervillous space must be opened up, presumably by trophoblast, intravascular trophoblast migration has not been seen in decidual veins.

It is not clear at what point maternal blood flow into the intervillous space is established. The spiral arteries are putatively opened up at 28 to 30 days postconception, but whether the blood flow is anything more than a seepage at this stage is debated. It seems that a through circulation is not established until about 12 weeks' gestation (Burton et al. 1999). In the final stages of transformation of the spiral arteries into fully developed uteroplacental arteries, the trophoblastic cells become entirely incorporated into the wall of the vessel and there is no longer direct contact with intraluminal trophoblast, the endothelial lining being reconstituted (Khong et al. 1992). During the third trimester the affected vessels undergo progressive distention, resulting in a series of dilated, funnel-shaped, tortuous vessels opening into the intervillous space (Fig. 3.6). It is assumed that the fully developed uteroplacental arteries lose their ability to respond to vasomotor influences because of the loss of their musculoelastic tissue and probably their autonomic nerve supply. There is certainly a significant drop in peripheral resistance at the opening of the uteroplacental arteries into the intervillous space allowing greater conductance and, hence, an increased blood flow at a low pulse pressure into and through the intervillous space. In this way the tenfold increase in blood supply required by the fetoplacental unit in the third trimester can be accommodated.

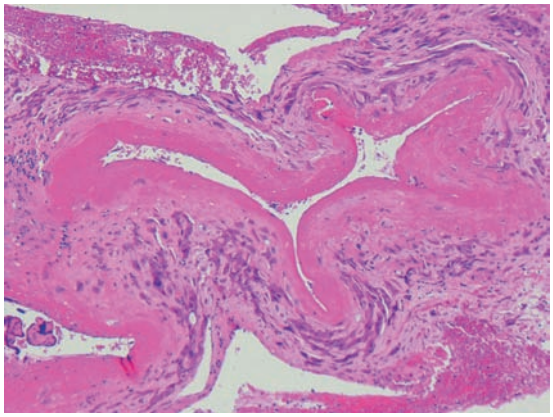

Figure 3.6. Uteroplacental artery at term with dilated lumen and fibrinoid matrix within the wall, surrounded by interstitial trophoblast.

Indications for Placental Examination

While some advocate examination of all placentas by a pathologist, a triage of placentas is realistically necessary so as not to overwhelm the diagnostic pathology service. Guidelines are available for indications for placental examination, although the interests of the local obstetrics and pathology departments may influence which placentas are sent for pathological examination (Langston et al. 1997). It is a good idea to incorporate a system where those placentas that are not sent for pathological examination can be stored in a refrigerator for about 1 week so that the placenta of the seemingly healthy infant who experiences early postnatal problems can be retrieved for examination.

Maternal indications for pathological examination include diabetes mellitus, preeclampsia, premature rupture of membranes, prolonged rupture of membranes, preterm delivery, placenta previa, postmaturity, and poor previous obstetrical history. Fetal indications include stillbirth, neonatal death, multiple gestation, prematurity, intrauterine growth restriction, congenital anomalies, hydrops, meconium-stained liquor, low Apgar scores, admission to neonatal intensive care unit, abnormal fetal cardiotocography, and suspected infection. Placental indications are placental abruption, infarcts, placenta previa, abnormal calcification, and any abnormal appearance of placenta, cord, or membranes. Despite the clear guidelines, placentas may not be submitted for examination or, conversely, may be submitted for pathological examination when there are no indications and unlikely to yield any useful information (Spencer and Khong 2003).

It is important that close liaison is established between the pathology department and the delivery suite and obstetrics unit so that the placenta, umbilical cord, and membranes are received in an optimal state for histopathological assessment. Use of standard forms listing indications and in-service education of obstetric staff can result in good compliance with guidelines (Kent and Dahlstrom 2006).

Multiple Pregnancy

Placentation in multiple pregnancy has been reviewed at length in some texts and monographs (Strong and Corney 1967; Boyd and Hamilton 1970; MacGillivray et al. 1975; Baldwin 1994; Fox 1997; Benirschke and Kaufmann 2000; Kraus et al. 2004) and is detailed in Chapter 12.

Abnormalities in Development and Placentation

There is a wide variation in placental shape, which has no effect on the outcome of pregnancy. Accessory lobes occur in 3% to 8% of pregnancies and are the result of non-involution of chorion laeve in the membranes. They are usually of no clinical significance except when retained in utero after delivery or associated with vessels running a velamentous course and situated near the cervical os when there is danger of vessel rupture during labor and fetal exsanguination. Bilobate placentas are uncommon and are not associated with any effect on perinatal morbidity. Fenestrate placenta with central absence of villous growth is very rare and has been reported in a case with triploidy (Linhart et al. 2004).

Extrachorial placentation where the chorionic plate from which the villi arise is smaller than the basal plate, that is, where the transition from villous to nonvillous chorion takes place within the circumference of the fetal surface of the placenta, is present in 24% of pregnancies (Fox 1997). Where the membranes form a flat ring comprising

only amnion and chorion with fibrin, the placenta is classified as circummarginate, and this is without clinical significance. Where the transition is raised and contains decidual tissue, ghost villi, functioning villi, and blood clot, the placenta is classified as circumvallate. The totally, but not the partially, circumvallate placenta is associated with low birth weight and a high rate of threatened abortion and premature onset of labor (Fox 1997). It is not uncommon to find partially circumvallate or circummarginate placentas or a mixture of both. Circumvallate placentas are found more commonly in multigravida.

Placenta previa occurs when implantation is in the lower uterine segment, lying in advance of the presenting fetal part. This may be total, where the placenta covers the internal os completely; partial, where part of the internal os is covered; or marginal, where the placental edge just reaches the internal os. The incidence of this disorder is approximately 1 in 250 births, the majority occurring in parous women. It is associated with severe antepartum hemorrhage, and delivery by cesarean section is required. Placenta previa is a clinical diagnosis and cannot usually be diagnosed by examination of the placenta, although the finding of the site of rupture of the membranes at the placental edge is supportive evidence.

Placenta accreta occurs when there is direct apposition of placental villi to myometrium. This may be complete or partial. It has been argued that many pregnancies necessitating manual removal of placenta represent minor degrees of the condition. Predisposing factors are placenta previa, previous uterine surgery including curettage and cesarean section, and previous manual removal of placenta. The reported incidence of placenta accreta has increased in conjunction with increasing cesarean rates and advanced maternal age (Wu et al. 2005). Using histopathological and strong clinical criteria, the incidence was found to be as high as 1 in 533. It is associated with antepartum hemorrhage, uterine inversion and rupture, and postpartum hemorrhage. A missing piece of tissue from the maternal surface may indicate a degree of accreta. Histologically, even in fragmented tissues following manual removal of the placenta, the diagnosis may be confirmed by the finding of myometrial fibers

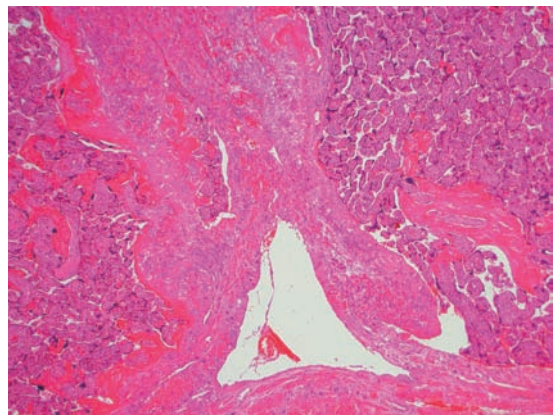

Figure 3.7. Placenta accreta showing myometrial fibers in basal plate and placental septum.

pulled up into placental septa (Fig. 3.7) or immediately subjacent to the Nitabuch's fibrinoid layer without an intervening layer of decidua basalis (Khong and Robertson 1987). The finding of myometrial fibers in the basal plate, however, does not necessarily correlate with symptoms of placenta accreta (Khong and Werger 2001). A study of 310 pregnancies between 1990 and 2000 complicated by placenta accreta found an increased incidence of preterm delivery and small-for-gestational-age infants (Gielchinsky et al. 2004).

In placenta membranacea there is persistence of villous growth over the whole surface of the placental membranes. It is very rare (1 in 3300 to 1 in 21,500 deliveries) and said to be associated with low birth weight and recurrent bleeding in the first and second trimesters, often leading to spontaneous abortion or premature labor, retained placenta, and postpartum hemorrhage. Good outcome has been described also (Ahmed and Gilbert-Barness 2003).

Placental Macroscopic Abnormalities

Many of the pathological abnormalities that are apparent on naked eye examination of the placenta are related to disturbances in either maternal or fetal circulations. These abnormalities may be difficult to distinguish from each other and may require histological examination for identification.

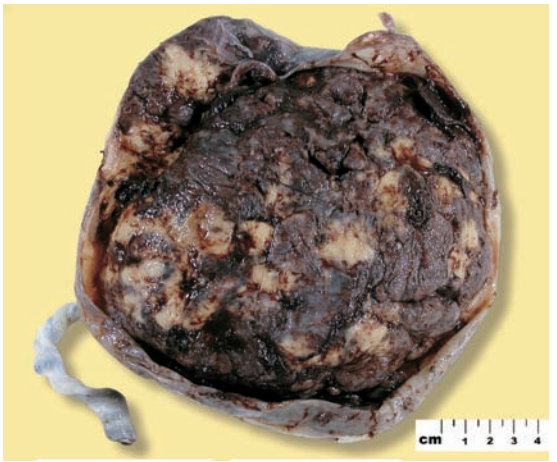

Figure 3.8. Maternal surface of placenta showing multiple areas of infarction.

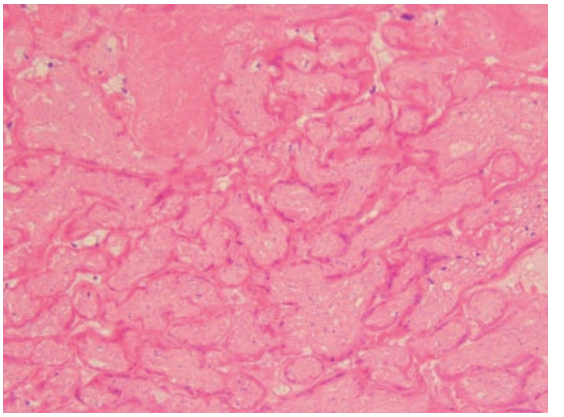

Figure 3.10. Old placental infarct. The villi are crowded and ghost like; the trophoblast has undergone necrosis.

Infarction

Single small infarcts are common while numerous multiple infarcts occur most commonly in placentas from preeclamptic women. Cessation of the fetal circulation following fetal demise is not accompanied by discrete areas of infarction within the placenta. A fresh infarct seen in the cut surface of the placenta is dark red and moderately soft. As the infarct ages it appears brownish then yellow and finally white and firm (Figs. 3.8 and 3.9). The histological appearance of an infarct is characterized by villous crowding, narrowing of the intervillous space, congestion of the fetal vessels, and pyknosis of the syncytiotrophoblast nuclei. As the infarct ages, villi undergo necrosis and an old infarct consists of "ghost" villi (Fig. 3.10).

It is generally accepted that infarcts occupying less than 10% of the parenchyma are insignificant. Infarction of a larger proportion of the placenta is associated with a high incidence of intrauterine growth restriction and intrauterine death. Infarction is not the primary cause of fetal problems but an indication of reduced maternal blood supply to the placenta (vide infra) (Fig. 3.11), and the use of the term *uteroplacental insufficiency* has been advocated (Wigglesworth 1964).

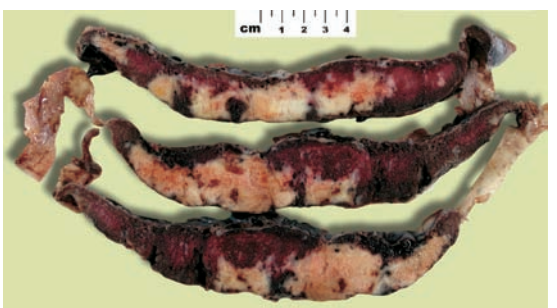

Figure 3.9. Placental slices showing areas of infarction, with two areas of more recent infarcts in the upper slice.

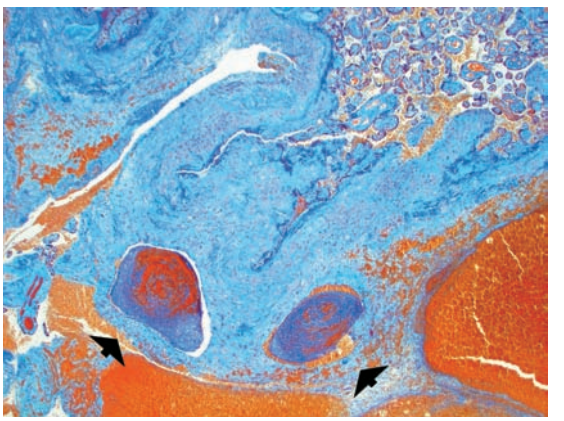

Figure 3.11. Thromboses in the maternal vessel in the basal plate (arrowheads) (Martius scarlet blue stain, MSB stain).

Hematoma: Retroplacental and Marginal

Retroplacental hematomas were described in 4.5% of placentas and were three times more common in placentas from preeclamptic women (Fox 1997). The source of the hemorrhage may be arterial, venous, or both. Retroplacental hematomas are often accompanied by adjacent placental infarction and decidual necrosis.

Hematomas vary in size from less than 10 mm, only apparent when the placenta is sliced, to large lesions that may involve much of the maternal surface. On the cut surface of the placenta the hematoma may be seen bulging into placental tissue, compressing it, and causing infarction (Fig. 3.12). The clot, when newly formed, is soft and red in color and may become separated from the placenta during delivery. Careful inspection of the maternal surface reveals a depression at the site of hematoma formation, and adherent strands of fibrin give the surface a dull rough surface. This enables distinction from insignificant blood clot, which may be delivered with the placenta but which does not indent the maternal surface. The histological appearance of hematomas is age related. Fresh clots consist entirely of red blood cells. With age, there is increasing fibrin deposition, degeneration of red blood cells, and infiltration of the basal plate by polymorphonuclear leukocytes and hemosiderin-laden macrophages. The effects of a retroplacental hematoma are dependent on the size and on the integrity of the underlying maternal blood supply. In an uncompromised pregnancy, a large lesion involving the central area and up to 40% of the maternal surface will cause fetal embarrassment, while smaller retroplacental hematomas may prove equally embarrassing in pregnancies complicated by preeclampsia, a situation where the maternal blood supply is diminished.

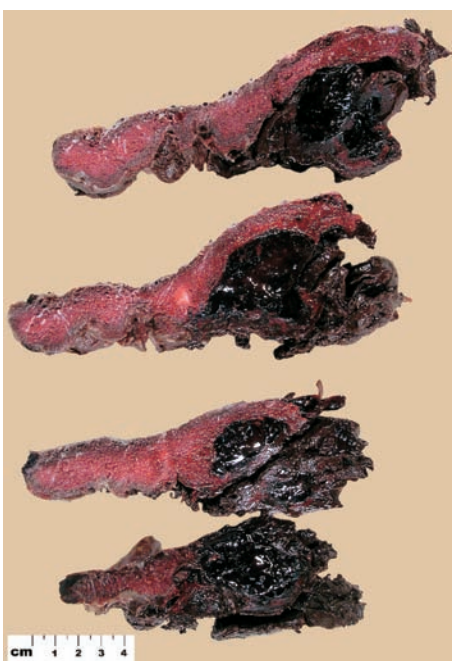

Figure 3.12. Retroplacental hematoma with excavation of the clots to leave indented placental parenchyma.

Peripheral or marginal hematomas form at the lateral margin of the placenta and may spread onto the maternal surface but not compress it. An association has been noted among premature delivery, premature rupture of the membranes, and peripheral placental hemorrhage (Harris et al. 1985).

Abruptio Placenta

Abruptio placenta implies a premature separation of the placenta from the maternal surface with decidual hemorrhage. The overall incidence of abruptio placenta is about 0.5%, and a history of abruptio placenta increases the risk of a similar episode in a subsequent pregnancy by 10-fold (Kraus et al. 2004). Cigarette smoking and cocaine use have been associated with abruptio placenta, presumably as a result of decidual necrosis. The incidence of abruptio placenta is increased in preeclampsia, diabetes mellitus, multiple births, short umbilical cord, and velamentous cord insertion (Ananth et al. 2005; Salihu et al. 2005). However, in many cases no cause of placental abruption can be identified. Infants born after abruption are significantly smaller-for-gestation than controls. Most studies report a perinatal death rate following abruption at approximately 50%, related to the strong association with preterm birth, and, paradoxically, is higher for singleton than multiple births (Salihu et al. 2005).

If placental separation takes place over a long period of time, in stages, the fetus may survive but suffers from disruption of its maternal blood supply. This prolonged sequence of events results

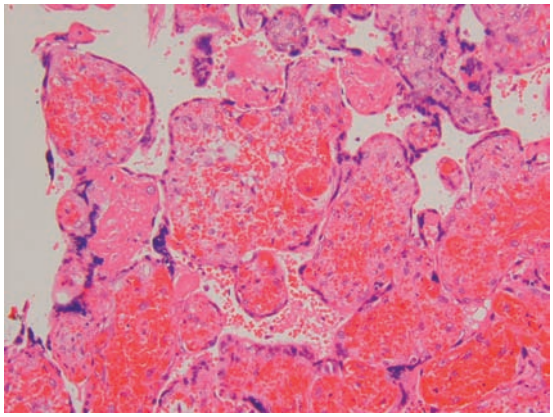

Figure 3.13. Intravillous hemorrhage in congested villi in placental abruption.

often in retroplacental hematoma. The acute placental separation, on the other hand, occurring immediately prior to delivery or causing sufficient fetal distress as to warrant emergency delivery, may not be recognized reliably by the pathologist as no gross evidence may be present; there may be no adherent blood clot or indentation of the placental parenchyma. However, there may be histological evidence with intense congestion and dilatation of the fetal vessels of the affected area. In one study, chorionic villous hemorrhage was seen in half of cases of retroplacental hemorrhage (Mooney et al. 1994) (Fig. 3.13). Intramyometrial segments of spiral arteries lacked physiological changes in 60% of placenta abruptio cases, whether hypertensive or not. Bleeding in the myometrium due to the presence of abnormal vessels, not clearly identified as arteries or veins, was suggested (Dommisse and Tiltman 1992).

Subchorial Thrombosis

Accumulation of blood between the chorionic plate and underlying villous parenchyma protruding into the amniotic cavity may be identified in midtrimester missed abortions. Slicing shows a mass of red thrombus dissecting the chorionic plate from the underlying villi. The thrombus is maternal in origin and histologically consists of villus-free laminated thrombus. The belief that they occur only after fetal death has been discounted by the finding of similar lesions in live-born infants. Subchorial hematomas can be identified antenatally by ultrasound, and it appears that its incidence is the same in the first and second trimesters, but those in the second trimester are often larger and may be associated with an increased risk of preterm delivery (Pearlstone and Baxi 1993).

Intervillous Thrombosis

Thrombus in the intervillous space is more frequent as pregnancy progresses and is seen in 36% of placentas from uncomplicated term pregnancies (Fox 1997). Grossly, they are roughly spherical and as the thrombus ages it changes in color from soft to firm and from dark-red to brown and eventually becomes a laminated white lesion. Fetal and maternal cells are seen in the thrombi, and the increased frequency near to term, when fetomaternal hemorrhage is most frequent, suggests that thrombosis may occur at the site of fetomaternal hemorrhage. Intervillous thrombosis and infarcts often occur together, and maternal thrombophilias has been suggested as a common pathogenetic pathway (Becroft et al. 2004) (Fig. 3.14).

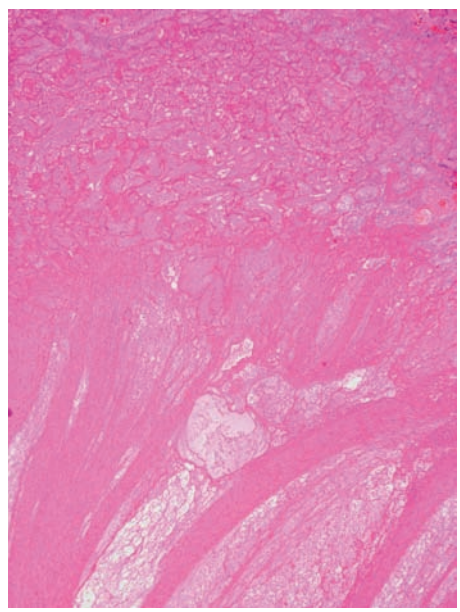

Figure 3.14. Area of intervillous thrombosis with contiguous infarction.

Perivillous Fibrin Deposition and Maternal Floor Infarction

Some deposition of fibrin can be seen around villi on histological examination of all placentas at term and may be associated with some villous atrophy. It is of no clinical significance.

Massive perivillous fibrin deposition and maternal floor infarction are two closely related entities. Massive perivillous fibrin deposition, sometimes affecting up to 30% of placental parenchyma, can be detected macroscopically as irregular white areas and may be indistinguishable from infarcts or may be seen as a meshwork of whitish swaths of agglutinated villi (Fig. 3.15). Grossly, the lesion is most frequently seen in the peripheral area of the placenta but may be situated centrally. It is thought that turbulence of blood in the intervillous space leads to fibrin deposition. Histologically, the lesion consists of villi that are widely separated by fibrin. The syncytiotrophoblast becomes degenerate but the cytotrophoblastic cells may proliferate. The entrapped villi become secondarily infarcted (Fig. 3.16). Maternal floor infarction is a misnomer, as the lesion is not due to an ischemic process but rather to an excessive fibrin deposition in the basal plate. Grossly, the furrows of the lobes are lost as the maternal surface becomes encased within fibrin, giving the placenta a ligneous feel. Histologically, maternal floor infarction is characterized by a thickened layer of perivillous fibrin deposition involving villi at the maternal surface, which subsequently become necrotic and avascular.

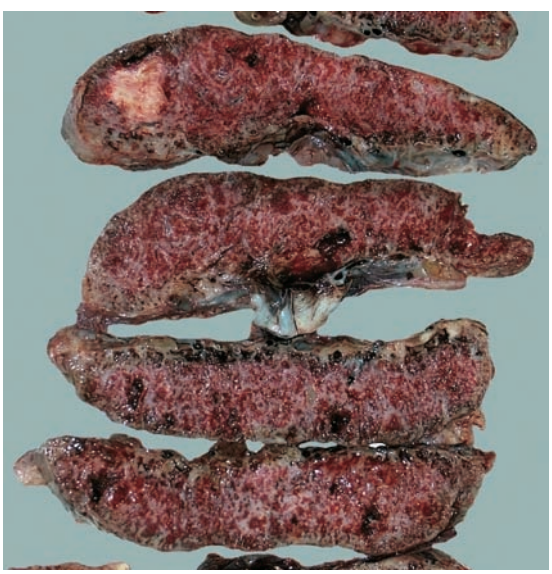

Figure 3.15. Slices from a placenta with massive perivillous fibrin deposition showing characteristic mesh of affected villi with cystic areas of normal villi.

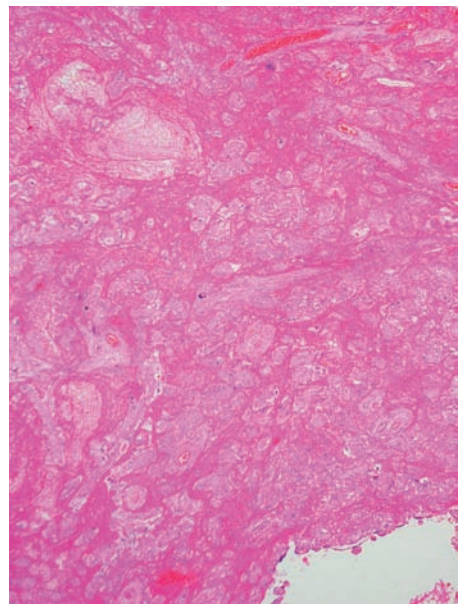

Figure 3.16. Massive perivillous fibrin deposition. The villi are encased within, and separated from each other by, fibrin. Lower right shows normal intervillous space, corresponding to the cystic spaces is seen macroscopically.

Massive perivillous fibrin deposition can affect up to 20% to 30% of the villous population without significant complications. Lesions affecting over half the villous tissue are rare and are associated with fetal distress and fetal growth restriction. The apparent anomaly whereby infarcts affecting 10% of the villous parenchyma are significant but massive perivillous fibrin deposition affecting up to 30% is not can be explained by the underlying maternal uteroplacental blood supply. Infarction normally occurs in the setting of an already compromised maternal blood supply, while massive perivillous fibrin deposition usually occurs in the setting of a good maternal uteroplacental blood supply (Fox 1997).

Maternal floor infarction and massive perivillous fibrin deposition are associated with fetal death and intrauterine growth restriction and

may recur in subsequent pregnancies. The recurrence rate appears to be about 20% but is based on small numbers in the literature (Khong 2002). The association with growth restriction is about 30% and seems to be more strongly correlated with massive perivillous fibrin deposition than with maternal floor infarction (Katzman and Genest 2002).

Chorangioma

Chorangiomas are hemangiomas occurring within villi and they occur in 1% of all placentas (Fox 1997). Most are small, single, discrete, and intraplacental; because of their characteristic red color they may be indistinguishable from fresh infarcts on gross examination. Small chorangiomas are clinically insignificant. Larger chorangiomas (larger than 50 mm in diameter) may be intraplacental and elevate the fetal surface. They may lie on the maternal surface or within membranes, or may be attached to the placental disk by a vascular pedicle (Fig. 3.17). They may be visualized antenatally by ultrasound examination and may be an infrequent cause of elevated maternal serum α-fetoprotein (Khong and George 1994). Large or numerous chorangiomas may affect the fetus by inducing high-output cardiac failure due to arteriovenous anastomoses. Polyhydramnios, the cause of which is unclear, is reported in a high proportion of large chorangiomas. Fetal hydrops, anemia, thrombocytopenia and cardiomegaly, premature labor, and antepartum hemorrhage have been described. Hemangiomas of the fetus are occasionally found in association with large chorangiomas.

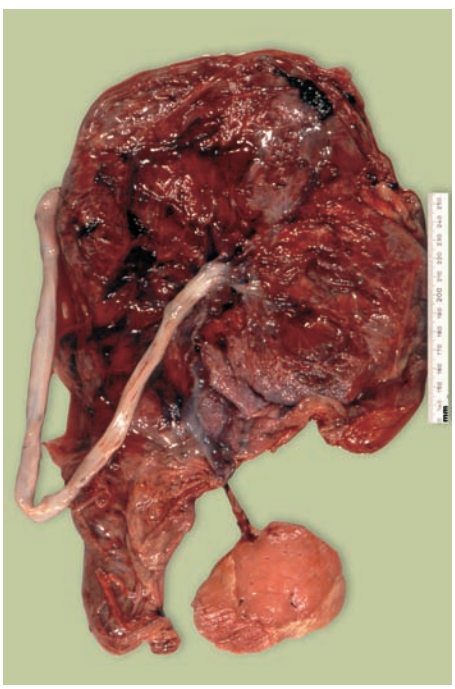

Figure 3.17. Chorangioma attached to the fetal surface of the placental by a vascular pedicle.

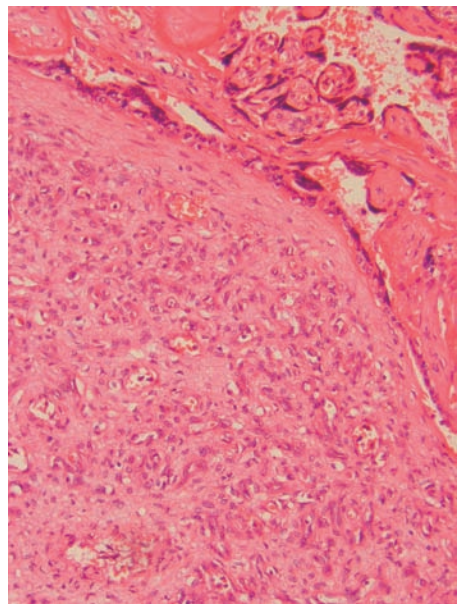

Figure 3.18. Chorangioma showing numerous vascular channels with a normal syncytiotrophoblast layer.

Large chorangiomas are usually purplish-red, encapsulated, of variable shape, and frequently divided by fibrous septa. Histologically, they resemble a capillary hemangioma and/or have a predominant spindle-cell pattern (Fig. 3.18). Degenerative changes such as necrosis, calcification, hyalinization, and myxoid change are frequently present. The trophoblastic mantle may be hyperplastic, but this appearance does not seem to confer any different clinical significance to the common variety chorangioma (Khong 2000; Ogino and Redline 2000). The cases previously described as chorangiocarcinoma (Jauniaux et al. 1988) are best termed as chorangiomas with trophoblastic hyperplasia (Khong 2000) (Fig. 3.19). Maternal and fetal outcome appear normal.

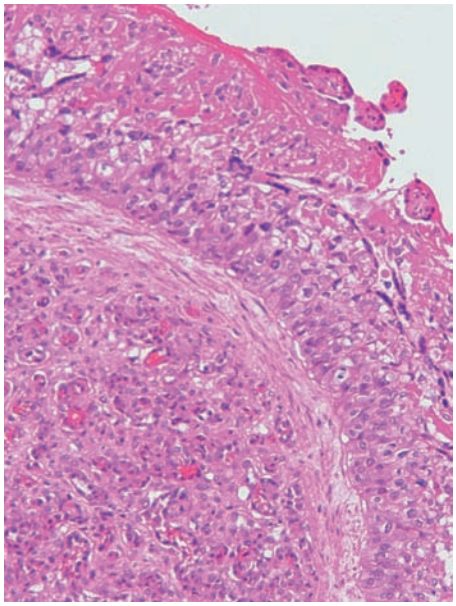

Figure 3.19. Chorangioma with trophoblastic hyperplasia.

There have been reports of multiple nodules of hemangiomatous tissue scattered throughout the placenta, a condition referred to as chorangiomatosis, which should be distinguished from chorangiosis.

Fetal Stem Artery Thrombosis

Fetal stem artery thrombosis is usually a single lesion and produces a sharply defined zone of avascular villi (see also Fetal Thrombotic Vasculopathy and Thrombophilia, below). The lesion is found in 4.5% of placentas from term uncomplicated pregnancies but in 10% of placentas from diabetic women. A relatively high incidence (14%) of this lesion is also seen in placentas from stillbirths. An association between fetal artery thrombosis and maternal coagulopathies has been noted. Most placentas with fetal artery thrombosis have normal outcomes with healthy infants. Placentas with multiple fetal artery thromboses, affecting more than 30% of the parenchyma, are associated with an adverse perinatal outcome, including stillbirth. Multiple extensive fetal artery thromboses are rare, however. It has been suggested that thrombosis within the placenta may be a marker for thrombosis elsewhere in the fetal vasculature, and there may be brain lesions secondary to cerebral thrombosis or later evidence of cerebral palsy (Kraus and Acheen 1999).

The lesion is easy to identify as a pale triangular area with its base in the chorionic plate. Histological examination shows a sharp division between avascular and uninvolved villi and no villous crowding is observed. A large thrombosed stem artery can be identified at the apex of the lesion (Fig. 3.20). Organization and recanalization of the thrombus may be seen. Vessels distal to the occlusion show progressive fibromuscular sclerosis and the connective tissue is fibrous with a hyalinized appearance (Fig. 3.21). There may be linear deposition of hemosiderin on the trophoblastic basement membrane of many avascular villi and also deposition in the stroma (McDermott and Gillan 1995). There is an excess of syncytial knots but no cytotrophoblast proliferation.

Other

- Visible calcification of the placenta, which may be extensive, is of no clinical significance, being found equally frequently in fresh stillbirths and in live-born infants.

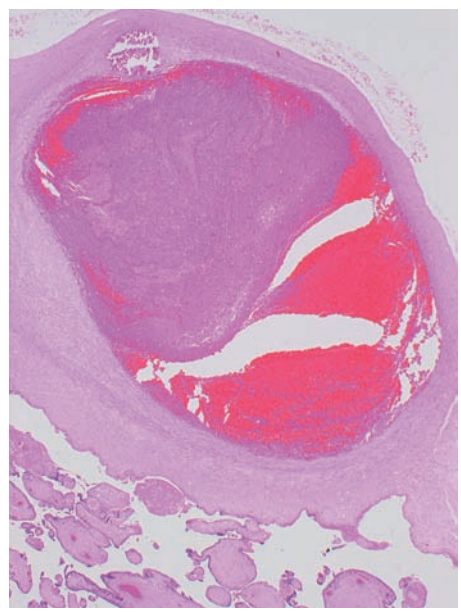

Figure 3.20. Thrombosis of chorionic plate vessel with collapse of villous vessels in downstream villi.

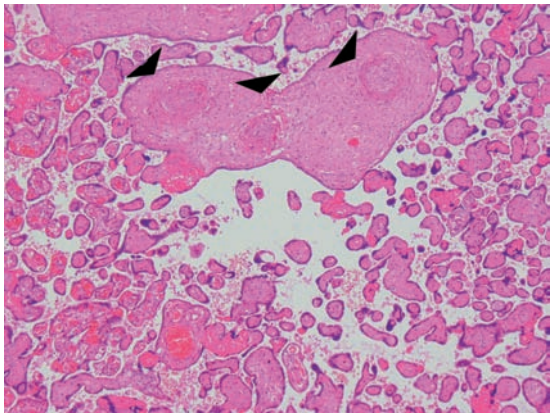

Figure 3.21. Fetal artery thrombosis. Tracts of avascular villi, on the right, downstream from occluded stem vessels (arrowheads).

- Septal cysts are oval or round cysts formed by a smooth thin glistening membrane and are often located in the subchorionic zone. They are of no clinical significance.
- A laminated, white plaque of fibrin devoid of any entrapped villi is sometimes seen on the undersurface of the chorionic plate. Subchorionic fibrin plaques are insignificant clinically. An unconfirmed finding is that an absence of subchorionic fibrin is associated with an increased incidence of postnatal mental handicap (Naeye 1990, 1992); the suggestion is that diminished fetal activity or movement as a result of neurological impairment in utero leads to reduced trauma of the chorionic plate and subsequent reduced or absent fibrin plaque formation.
- Placental mesenchymal dysplasia is a recently recognized placental vascular malformation. Some 50 cases have been reported. There are numerous dilated and thick-walled vessels on the fetal chorionic surface that may extend into the intraplacental parenchyma. The vessels are variously described as being thick walled and varicose or aneurysmal. One third of reported cases were associated with Beckwith-Wiedemann syndrome. The pregnancies are often complicated by prematurity and intrauterine growth restriction. There is a predominance of female fetuses associated with placental mesenchymal dysplasia (Khong 2004).

Placental Microscopic Abnormalities

Villous Maturity

Villous immaturity is seen in placentas from diabetic women, maternofetal rhesus incompatibility, and in syphilis, anencephaly, and Down syndrome. Accelerated villous maturity is seen in a proportion of placentas from immature, prematurely delivered infants and in preeclamptic pregnancies.

Syncytial knots, which are clumps of syncytial nuclei that protrude into the intervillous space from the surface of the villi, are seen increasingly in the later stages of pregnancy. There is a tendency for increased syncytial knot formation in preeclampsia, essential hypertension, and diabetes mellitus. The literature is now imbued with the concept of excessive syncytial knot formation (Tenney–Parker changes) and accelerated villous maturation as being markers of uteroplacental ischemia, but this is not convincingly proven (Fox 1997). Furthermore, assessing villous maturity is fraught with observer reproducibility errors (Khong et al. 1995).

Villous maturity is also confused with placental aging. Placentas from postterm pregnancies do not show any villous abnormalities on histology or morphometry (Larsen et al. 1995).

Placental Edema

Villous edema is seen in 11% of unselected term placentas, with significant associations with fetal and neonatal death.[49] The placenta is often edematous in those conditions that give rise to fetal hydrops, particularly when hydrops is the result of chronic fetal anemia. In these conditions severe fetal edema is usually accompanied by a similar degree of placental edema, but this is not always the case and placental edema may be the more striking change, particularly in rhesus incompatibility. Some abnormalities that produce fetal hydrops are rarely accompanied by placental edema. Conversely, the placenta may be hydropic while the fetus is normal, as in congenital nephrotic syndrome.

The hydropic placenta is pale, friable, and bulky, often weighing more than 1 kg. Fluid exudes from the cut or damaged surface, and intervillous

thrombi or septal cysts are frequently seen. Because of its friability, manual removal of the placenta is often necessary and postpartum hemorrhage may occur. Histological examination reveals edema affecting immature intermediate villi; the change may be focal or uniform. Hofbauer cells and cytotrophoblast appear prominent (Shen-Schwarz et al. 1989). Villous syncytiotrophoblast is usually normal, and thickening of the basement membrane may be seen. Fibrinoid necrosis of the villi is frequently seen. When there is severe fetal edema, focal erythropoiesis is present within fetal capillaries. The overall impression in this condition is one of crowding of immature and edematous villi. Striking differences in villous appearance are seen in different areas of the same placenta, and focal villous hypercellularity is often present.

Acute Atherosis

Acute atherosis is characterized by fibrinoid necrosis of the arterial wall with a perivascular lymphocytic infiltrate and the presence of lipid-laden macrophages within the wall and lumen of the artery (Fig. 3.22). The lipid-laden macrophages appear late in the evolution of the lesion. The lesion is seen in arteries in the uterus that have not undergone physiological vascular changes of pregnancy and thus may be seen also in the decidua parietalis underlying the amniochorial membranes as well as unconverted spiral arteries and basal arteries in the decidua basalis and in the myometrium. A novel method of embedding the amniochorial membranes to detect acute atherosis by stacking the membranes has been described (Walford et al. 2005). Acute atherosis has been reported in preeclampsia and intrauterine growth restriction and has been claimed to be seen in normal pregnancies terminated in the first trimester, but this has been disputed by others. It is noteworthy that acute atherosis lesions immunolabel for lipoprotein [a], which is atherogenic and thrombogenic, explaining the frequent finding of thrombosis with acute atherosis (Meekins et al. 1994). The possibility of an immunological pathogenesis of the lesion is suggested by the morphological similarity of this lesion to that seen in allograft rejection (Khong 1991) and by clinical parameters (Khong et al. 1987). Immunoglobulin M (IgM) and complement (C3) have been found in acute atherotic lesions similar to the findings in atherosis-like lesions from rejected renal and cardiac transplants, leading to speculation that acute atherosis is primarily an immunologically determined vasculopathy (Labarrere 1988). Development of acute atherosis may be due to complement-fixing immune complexes along with a T cell or natural killer maternal aggression on fetal tissues (Labarrere 1988) but the perivascular cell infiltrate is similar to that seen around vessels with physiological changes and those vessels with neither physiological changes nor acute atherosis (Khong et al. 1987).

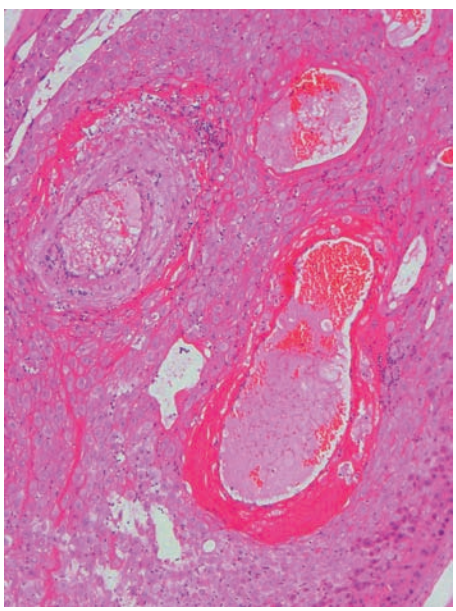

Figure 3.22. Acute atherosis. Arterial sections show fibrinoid necrosis, lipid-laden macrophages, and a scanty perivascular lymphocytic infiltrate.

Teratoma

Placental teratomas are rare tumors of disputed histogenesis. Some believe them to be a separate entity, while others believe that they are extreme examples of fetus amorphus. They are histologically benign with complete disorganization of its various structures and an absence of an umbilical cord. They are clinically insignificant.

Chorangiosis

The lesion called chorangiosis is defined as the presence of 10 villi each with 10 vascular channels in 10 or more noninfarcted and nonischemic zones of at least three different placental areas when examined with a × 10 objective (Altshuler 1984). It is predominantly a lesion of terminal villi (Ogino and Redline 2000). The incidence in normal uncomplicated pregnancy was not evaluated. Chorangiosis was seen in 5.5% of 1350 placentas examined for a limited defined range of maternal and fetal disorders (Altshuler 1984). Since these placentas had been examined because of maternal reproductive failure, intrauterine infection, dysmaturity, congenital abnormalities, diabetes, or erythroblastosis, it is not surprising that chorangiosis could be associated with incidences of neonatal death and major congenital malformations as high as 39% and 42%, respectively.

Fetal Thrombotic Vasculopathy and Thrombophilia

Thrombotic or occlusive lesions within the fetal circulation are not confined to fetal arteries. Accordingly, it may be preferable to use the term *fetal thrombotic vasculopathy,* which encompasses fetal artery thrombosis, hemorrhagic endovasculitis, fibromuscular sclerosis, and fibrinous vasculosis (Redline et al. 2004). The grossly obvious wedge-shaped tracts of avascular villi have been described above (fetal artery thrombosis). Occasionally, these uniformly avascular villi may be recognized on microscopy only. Intimal fibrin cushions may be recent or remote. The former are seen as deposition of fibrin or fibrinoid within the wall of larger fetal vessels, subendothelial or in the media (Fig. 3.23). Older lesions will show calcification (Fig. 3.24).

Obliterative fibromuscular sclerosis, seen commonly following collapse of fetal vessels after fetal death, is regarded as a lesion within the spectrum of fetal thrombotic vasculopathy. There may be organization and recanalization of thrombi with neutrophils or nuclear debris in the vessel walls. These features of villous stromal-vascular karyorrhexis have been termed hemorrhagic endovasculitis (Sander et al. 1986) (Fig. 3.25). This lesion has evoked controversy, and some believe that the lesion is not a true entity since it is seen following fetal death, and its morphological features may be the result of endothelial disintegration and degeneration of blood constituents (Fox 1997; Benirschke and Kaufmann 2000). Hemorrhagic endovasculitis is associated with chronic villitis and maternal hypertension and adverse outcomes in subsequent pregnancies and may be recurrent (Sander et al. 2005).

Various inherited thrombophilias are associated with neonatal encephalopathy and cerebral palsy and possibly with placental abruption, intrauterine growth restriction, preeclampsia, and

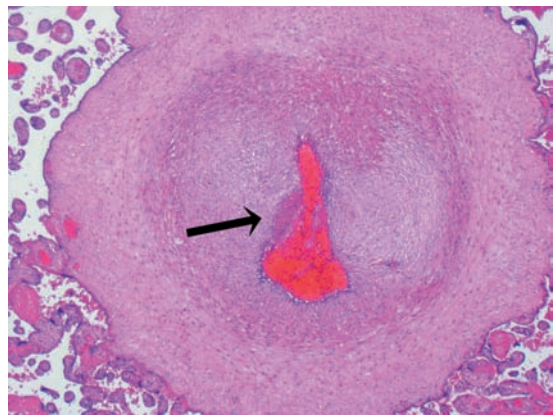

Figure 3.23. Intimal fibrin cushion (arrow) in a stem vessel also showing obliterative fibromuscular sclerosis.

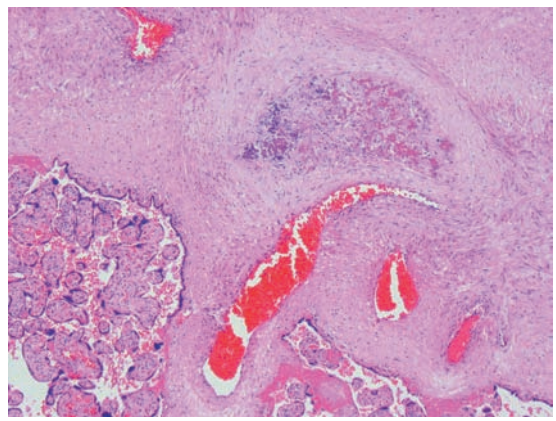

Figure 3.24. Calcified plaque within media in older intimal fibrin cushion.

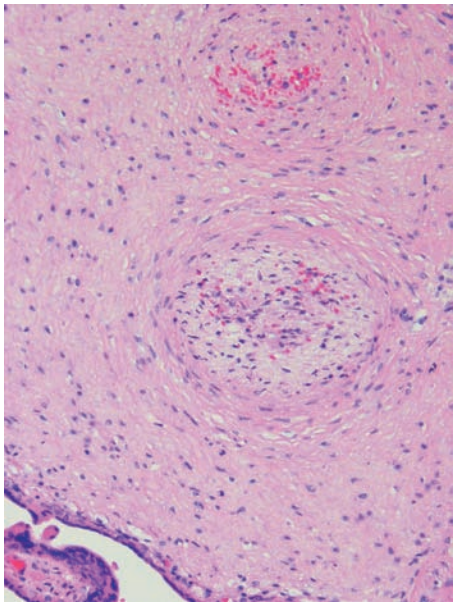

Figure 3.25. Hemorrhagic endovasculitis showing nuclear debris in vessel wall and recanalization.

intrauterine death (see Chapter 8) (Brenner and Kupferminc 2003). There does not appear to be any correlation between histological fetal thrombotic vasculopathy and most of the currently known inherited thrombophilias (Ariel et al. 2004). It is possible that additional stressors, such as umbilical cord pathology, to thrombophilia are important contributors to fetal thrombotic vasculopathy (Redline et al. 2004).

Umbilical Cord

Cord Length

The length of the umbilical cord varies widely throughout gestation, being between 54 and 61 cm at term (Mills et al. 1983; Naeye 1985). Cord length is dependent on the stretch provided by fetal intrauterine motor activity (Moessinger et al. 1982). Accordingly, short cords may be a marker of abnormal fetal brain development, secondary to maternal ingestion of beta-blockers or intrauterine constraint, such as twin pregnancy, uterine abnormalities, or reduced amniotic fluid volume. An abnormally short cord is defined as being less than 32 cm, which is the minimum length considered sufficient to permit unrestricted vertex delivery. The incidence of abnormally short cords is between 0.4% and 0.9%. Fetal hypoxia may result from stretching with obstruction of vessels, placental separation, or cord rupture. Fetuses with a short cord were more likely to be small for gestational age or have fetal distress, while mothers were more likely to have a retained placenta (Krakowiak et al. 2004). An abnormally long cord is considered to exceed 100 cm; thus defined, it is found in 0.5% of cords. When defined as being more than 2 standard deviations (SDs) above the mean for 30,000 placentas, which corresponded to more than 70 cm, excessively long umbilical cords was found in 3.95% of cords (Baergen et al. 2001). Berg and Rayburn (1995) defined an abnormally long cord as being more than 80 cm in length and found this in 3.7% of cords. Long cords predispose to prolapse, cord knots, and fetal entanglement, and thus to fetal morbidity and mortality. Histologically, long cords were associated with nucleated red blood cells, chorangiosis, fetal vascular thrombi, intimal vascular cushions, and single umbilical artery (Baergen et al. 2001). The umbilical cord is often edematous when there is fetal or placental hydrops, in maternal diabetes, and in Beckwith-Wiedemann syndrome.

Single Umbilical Artery

Single umbilical artery may result from primary aplasia of one vessel or secondary atrophy. The incidence varies from 0.2% to 1.1% of births, but in perinatal autopsy series the incidence has varied from 2.7% to 12%. The incidence is higher in girls than in boys (Lilja 1991). In determining the number of umbilical vessels, naked-eye examination can be misleading, and histology should be the definitive method of ascertainment. It is also noteworthy that the cord must be sampled sufficiently distant from its placental insertion (at least 5 cm), as the two arteries may fuse into one trunk, giving rise to an erroneous diagnosis of a single umbilical artery. Fusion of umbilical arteries near the placental insertion is more common in females than in males (Fujikura 2003). Ultrasound diagnosis, while specific, is not highly sensitive. Single umbilical artery is associated with maternal diabetes and maternal smoking and with low birth weight and preterm births

(Lilja 1991). An increase in fetal malformation is reported in association with single umbilical artery in between 20% and 50% of cases. Frequently associated malformations are sirenomelia sequence, VACTERL complex (vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula and esophageal atresia, radial and renal anomalies, and limb defects), and anorectal atresia and esophageal atresia (Lilja 1992). A significant proportion of asymptomatic infants born with a single umbilical artery have occult renal anomalies but there is a lack of data regarding other organ systems (Srinivasan and Arora 2005). Single umbilical artery is seen more frequently in trisomy 13, trisomy 18, and Zellweger syndromes, and although reputed not to occur with trisomy 21 (Khong and George 1992), we have seen cases associated with trisomy 21 since our previous report.

Umbilical Cord Insertion

The umbilical cord may be inserted into the placental disk centrally, eccentrically, marginally (battledore), or velamentously (via the membranes). There is no association between the first three types of insertion and premature delivery or other abnormality of fetus or pregnancy. In velamentous insertion, the cord inserts into the membranes, and hence the vessels run unprotected for some distance in the membranes before insertion into the placenta (Fig. 3.26). Velamentous insertion carries the danger of vessel rupture during labor, especially if vessels run across the internal os (vasa previa), where the perinatal mortality rate has been quoted to be as high as 75% to 100% (Robert and Sepulveda 2003), although the complication occurs in only 2% of such cases (Quek and Tan 1972). Another potential complication is compression of the cord and vessels during delivery. An excess of fetal deformity, but not malformation or disruption, is described in association with velamentous insertion of the cord. The umbilical cord can also be inserted velamentously but be ensheathed within Wharton's jelly until it reaches the fetal surface; this interposition, or interposito velamentosa, is uncommon. The umbilical cord can be tethered at its insertion by an amnionic web (chorda) or free fold of amnion that can potentially restrict the blood flow when the cord becomes angulated with movement (Fig. 3.27).

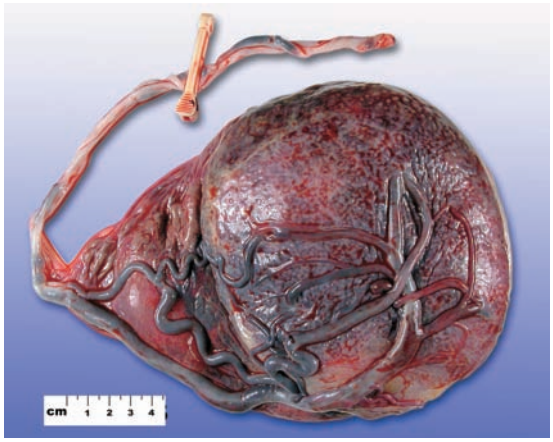

Figure 3.26. Velamentous cord insertion.

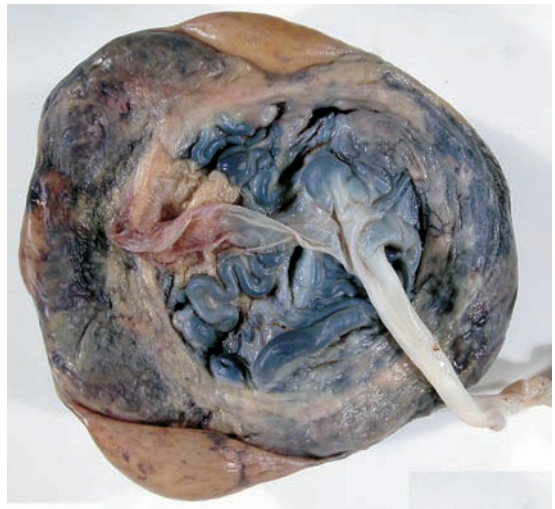

Figure 3.27. Amniotic web tethering umbilical cord.

Cord Knots and Entanglements

"False knots" of the cord are localized accumulations of Wharton's jelly or vascular dilatation producing asymmetrical cord expansion. They are insignificant.

True knots are present in between 0.1% and 2.1% of cords at delivery and were more frequent in long umbilical cords, in male fetuses, and in multiparous women (Blickstein et al. 1987). While some authors have found no significant

association with fetal distress, Apgar scores, cord blood analysis, cardiotocographic abnormalities, or perinatal mortality and neonatal morbidity (Matorras et al. 1990; Maher and Conti 1996), others have found a significantly higher incidence of fetal distress and meconium-stained amniotic fluid among patients with true cord knots and a higher rate of antepartum fetal death among those fetuses (Hershkovitz et al. 2001; Airas and Heinonen 2002). The knots are significant only if tight enough to obstruct fetal circulation. The structure of the cord in respect to spiraling of vessels and the physical characteristics of Wharton's jelly diminishes the likelihood of obstruction of cord vessels by true knots. One possible exception is in the setting of an infant with a decreased cord diameter, as may be seen in a growth-restricted infant. An inverse relationship between cord diameter and venous perfusion pressure in the presence of a true knot has been found (Chasnoff and Fletcher 1977). Knots that have been present for some time produce grooving and kinking of the cord, with localized loss of Wharton's jelly, constriction, and sometimes thrombosis of the vessels. Previously loose knots may tighten just before or during labor and cause asphyxia. The significance of such knots may be overlooked, but intrapartum death, fetal distress, or neonatal asphyxia could be ascribed to such knots if there is edema and congestion or thrombosis of vessels in the vicinity of a knot.

A long cord predisposes to fetal entanglement. The significance of cord entanglement is controversial, and it is commonplace to have it discussed at perinatal mortality and morbidity meetings. Cords around the neck are seen with increasing incidence with advancing gestation, being present in as many as a third of pregnancies at term (Clapp et al. 2003). A single coil is seen in 21.3% to 24.5% and two or more coils is seen in 3.4% to 3.8% (Carey and Rayburn 2003). Entanglement around a limb or torso is seen in 2% (Spellacy et al. 1966). These entanglements can produce sufficient kinking to affect the fetal circulation, especially if there is looping of the cord around itself. The arguments about the structure of the cord and its vessels in preventing vascular obstruction in true knots are equally applicable to cord torsion and entanglement. Nuchal cords are associated with an increased prevalence of variable fetal heart rate decelerations (Hankins et al. 1987). Adverse perinatal outcome is related to the number of coils around fetal parts (Spellacy et al. 1966). As many groups have reported an adverse effect of nuchal cord on birth weight as there are who have reported no such effect. Infants with a nuchal cord had a higher umbilical vein pO_2 and a lower artery pO_2 than those without a nuchal cord (Osak et al. 1997; Schaffer et al. 2005). The vexed question is when to ascribe significance to a cord complication: edema and congestion on one side of the alleged obstruction should be sought, while hemorrhage into Wharton's jelly and thrombosis of vessels and grooving of fetal parts increase the significance of cord entanglement.

Cord Coiling, Torsion, and Constriction

The umbilical cord is usually coiled as a result of active or passive rotation of the fetus, differential umbilical vascular growth rates, or hemodynamic torque. Generally, cords have a predominant counterclockwise twist, but some cords have a combination of clockwise and counterclockwise twists. Coiling appears to be established by 8 weeks' gestation, and loss of coiling has not been observed. There is more coiling at the fetal than at the placental end. The coiling index, as measured by counting the number of coils (coils/cm) is about 0.20. Values for normal pregnancy suggests that it may be slightly less, 0.17, but higher indices, 0.49 for placental end and 0.72 for the fetal end, have been reported (Blickstein et al. 2001; van Diik et al. 2002). A complete absence of coiling was found in 4.3% of cords, and this was associated with abnormal karyotypes and adverse perinatal outcome (Strong et al. 1993). Excessive loss may be associated with fetal loss or growth restriction (Machin et al. 2000).

Marked cord torsion may be distinguishable from the normal spiraling of the cord and may be observed at delivery of an uncompromised infant or following termination of pregnancy for fetal anomaly. It is when excessive cord torsion is observed following intrauterine fetal death that its significance is overemphasized. Following fetal death, fluid is lost from Wharton's jelly, leading to loss of turgor. Autolytic change in the cord may be accelerated in the 30 to 40 mm close to the umbilical cord insertion to the fetus. The general-

ized loss of turgor serves to make any torsion more apparent, and unopposed asymmetrical uterine action may produce further fetal torsion. Twists in the cord are often most apparent in the narrow segment of cord at the umbilical insertion to the fetus when torsion leading to cord constriction may be blamed for fetal demise. It is more likely that the twists collect in the constricted part of the cord as a purely mechanical phenomenon. Cord torsion per se is rarely the cause of fetal demise.

Constriction of the umbilical cord or a cord stricture usually occurs close to the fetal insertion and is much less commonly recorded elsewhere. In the constricted segment vessels are collapsed or contracted and the stroma appears dense. It has been suggested that such constrictions are the result of congenital absence or degeneration of Wharton's jelly; such a view is unproven. Cord constriction at the fetal end is exceptionally described with live birth when the constricted segment was very short. It is more likely that it is the result of more rapid autolysis occurring close to the fetus. Labarrere et al. (1985) described three rare cases where there was complete absence of Wharton's jelly around the umbilical cord arteries but present around the umbilical vein; these three cases were associated with perinatal death.

Localized constrictions elsewhere in the cord are the result of localized pressure. They are seen much more frequently in the presence of localized physical constriction, such as amniotic bands or significant cord knot, than as isolated lesions.

Amniotic Bands

A range of fetal abnormalities can be found with amniotic bands or adhesions to which the terms *amniotic band syndrome, amnion rupture sequence, amniotic deformity, adhesion and mutilation (ADAM) complex* have been applied. The abnormalities range from constriction rings, amputations, syndactyly, fusion defects of the cranium and face, and clefts. Two explanations have been advanced for the constellation of signs that cannot be readily explained by disordered development or teratogenic influence or environmental influence. In one, disruption of the amnion gives rise to bands causing a mechanical effect on the fetus. Alternatively, there may be a vascular compromise or a genetic or germ cell disruption. Immersing the placenta in water may allow the amniotic bands to be more easily seen. Histologically, the amnion is noted to be absent from the placental surface.

Localized Cord Swelling

Remnants of the allantoic and omphalomesenteric ducts may be found, typically located toward the fetal insertion of the cord, and ranging between 4 and 60 mm in size. The allantoic duct serves as a diversion for the fetal bladder, and its remnant is situated between the two umbilical arteries and generally lined by flattened and rarely by transitional epithelium. The omphalomesenteric duct connects the fetal yolk sac with the small intestine at the site of Meckel's diverticulum and would have atrophied during the 7th to 16th week. Remnants of the omphalomesenteric duct are located more peripherally in the cord than allantoic duct remnants and show an intestinal, often mucinous, lining. Abscess of an allantoic duct remnant has been described (Baill et al. 1989).

Cysts of the umbilical cord may arise from these remnants, usually situated at the fetal end of the cord. The lining of these cysts mirrors those of the duct remnants. These cysts are usually small and found by chance, but an occasional cyst may reach 40 to 50 mm in diameter when there is a risk of vascular tamponade. Amniotic inclusion cysts are uncommon. Pseudocysts, formed by cavitation as a result of mucoid degeneration of Wharton's jelly, lack an epithelial lining and contain clear mucoid material. Cysts detected on ultrasound antenatally appear to differ clinically if they are singular or multiple. Single cysts were associated with a favorable outcome, while multiple cysts were associated with chromosomal anomalies, such as trisomy 13 and 18, and with miscarriage (Ghezzi et al. 2003). Fetal abnormalities associated with umbilical cord cysts have included urachal anomalies and omphalocele.

Tumors of the cord are exceedingly rare. Teratomas of the cord are extremely rare.

Abnormalities of Umbilical Cord Vessels

Hematomas of the cord may be iatrogenic secondary to umbilical cord blood sampling,

amniocentesis, or intrauterine blood transfusion. Accordingly, it is important to seek such clinical information before describing them as spontaneous cord hematomas. Spontaneous hematomas in the cord arise from focal hemorrhage from an umbilical vessel, usually a vein. The majority appears to accumulate before the onset of labor. Their etiology is unclear. Although the perinatal mortality rate in infants whose cord contains a hematoma is about 40% to 50%, it is unclear whether the hematoma is responsible for the death and if so, how or whether the hematoma and death are related through a common cause (Fox 1997).

The prevalence of thrombosis of umbilical cord vessels is 1 in 1290 among prospectively examined placentas. There is a slight male predominance, and venous thrombosis occurs more frequently than thrombosis of one or both umbilical arteries. In a review, Heifetz (1988) did not find an association between cord thrombosis and perinatal mortality and morbidity and thought that thrombosis, when present, was related to additional umbilical cord abnormalities, obstetrical complications, or systemic fetal conditions that were likely cause of both the thrombosis and poor fetal outcome. Rarely, embolic spread of the thrombus from a cord thrombus to placental or fetal vessels may ensue.

Linear ulceration of the umbilical cord in association with congenital intestinal atresia has been described (Bendon et al. 1991). Severe hemorrhage from the cord ulceration into the amniotic cavity results in either severe neonatal asphyxia or intrauterine death. Additional abnormalities, including Hirschsprung's disease and an interstitial deletion of chromosome 13q (Khong et al. 1994) and trisomy 21 (Ohyama et al. 2000) have been described.

A closely related lesion, described by Altshuler and his colleagues (1992), is that of meconium-induced necrosis of the umbilical cord. The lesion is associated with prolonged meconium passage and there are meconium-laden macrophages in the Wharton's jelly associated with necrosis of the arterial wall (Fig. 3.28).

Marked segmental thinning of umbilical cord vessels was found in 1.5% of placentas. The media is reduced to only one or two layers of smooth muscle fibers. It is a focal lesion that shows a relatively abrupt transition from the normal to the abnormal segment. The lesion faces the cord surface in half the cases and affected the vein in 76% of cases and the arteries in the remaining 24%. All cases showed abnormalities of the vessels in the chorionic plate and primary stem villi. An association with congenital malformations was found and there was a high incidence of fetal distress. The origin of the lesion is unclear but may represent dysplasia of the media (Qureshi and Jacques 1994).

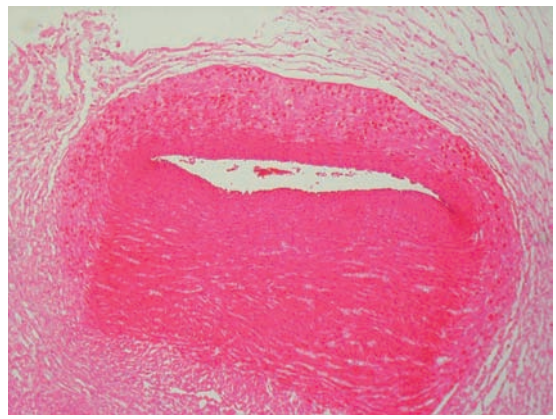

Figure 3.28. Meconium-induced myocytolysis of cord vessel wall.

Calcification of umbilical cord vessels is thought to be rare, and two different lesions have been described (Khong and Dilly 1989). In one there is complete calcification of the arterial lumen resulting in total obliteration, while in another, so-called sclerosing funisitis, there is sclerosis of the wall associated often with inflammation in the umbilical cord and its vessels, membranes and decidua, suggesting intrauterine infection (see section on necrotizing funisitis, page 92).

Hemangiomas, which may arise from either the umbilical vein or the artery, may give rise to cord hemorrhage, and is a known cause of elevated maternal α-fetoprotein levels. A high perinatal mortality rate due to premature delivery, fetal hydrops, cardiac failure, and intrauterine death is reported (Caldarella et al. 2003). Pseudocysts have been found in eight of 20 cases of umbilical cord hemangioma (Sondergaard 1994). Aneurysms of the umbilical vein or artery are rare: dissection or increase in size of the aneurysm may lead to compression of adjacent vessels. Three cases of aneu-

rysm of the umbilical artery have been described, each associated with a single umbilical artery and with trisomy 18 (Sepulveda et al. 2003).

Amniochorial Membranes

Amnion Nodosum and Squamous Metaplasia

Squamous metaplasia appears as slightly elevated granular deposits on the amniotic surface that are not easily displaced. Histologically, squamous metaplasia appears as foci of stratified epithelial cells with layers varying from 6 to 20 (Fig. 3.29). There is usually a sharp transition at the edge of the lesion from columnar to squamous epithelium. The lesion has no clinical significance.

Amnion nodosum is evident as numerous raised, shiny, grayish nodules measuring up to 5 mm lying on the amniotic surface of the placenta (Fig. 3.30). The nodules are easily dislodged, a distinguishing feature from squamous metaplasia of the amnion. Histologically, amnion nodosum consists of granular material containing fetal cells and debris with occasional hair fragments and may be covered by amniotic epithelium. The nodules derive from close contact between fetus and amnion and consist predominantly of vernix caseosa. The presence of amnion nodosum accompanies oligohydramnios from any cause so that an associated abnormality such as urinary tract obstruction or renal agenesis or a history of prolonged rupture of membranes should be sought. Squames may accumulate in the subchorionic space, presumably from cells shed into amniotic fluid, in prolonged amniotic fluid leakage not occurring over the cervical os (Bendon and Ray 1986).

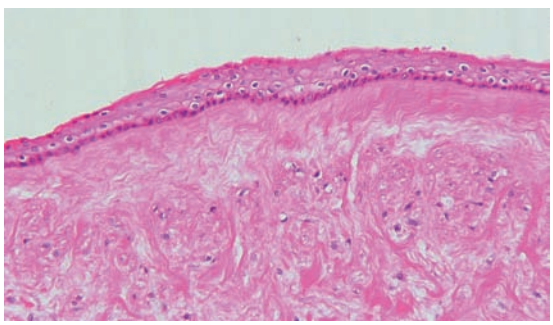

Figure 3.29. Squamous metaplasia of the amnion.

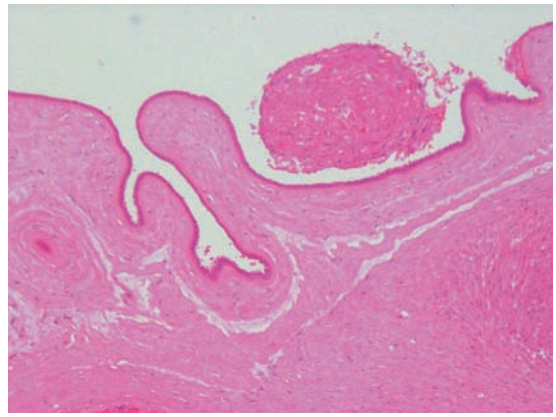

Figure 3.30. Amnion nodosum. Granular material containing squamae is seen on the surface of amnion.

Meconium Staining

Meconium staining of the membranes is seen in up to 20% of placentas but more commonly in postterm placentas. Meconium staining has traditionally been regarded as evidence of intrauterine fetal hypoxia and distress, but one critique concluded that the vast majority of fetuses with meconium staining of the membranes have not been subjected to a chronic hypoxic insult (Houlihan and Knuppel 1994). Indeed, only a handful of such infants have serious morbidity, principally associated with meconium aspiration syndrome. The view that meconium causes vasoconstriction of umbilical veins in the chorionic plate and subsequent cerebral palsy (Altshuler and Hyde 1989) is based on an in vitro study that needs to be confirmed.

Meconium staining can be identified by its uptake by macrophages in the membranes. However, not all pigment, in the membranes or chorionic plate, is meconium, and it has to be distinguished from hemosiderin. An in vitro study showed meconium-containing macrophages within the amnion within 1 hour of meconium exposure and within the chorion within 3 hours (Miller et al. 1985). Reliance of this timing of meconium exposure has been used in medicolegal circles, but it is questionable whether the in vitro conditions can be extrapolated to the in vivo

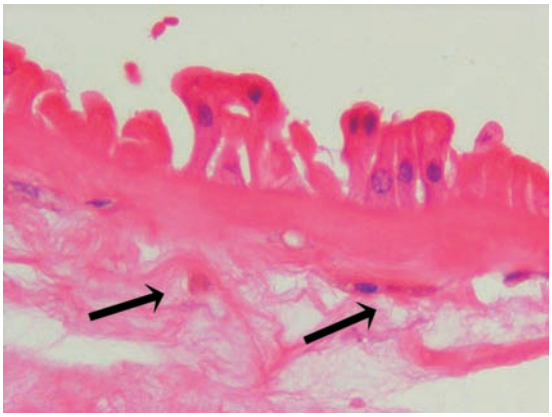

Figure 3.31. Meconium-induced changes in amnion with epithelial columnar metaplasia and meconium-containing macrophages within the amnion (arrows).

clinical situation. Prolonged meconium exposure results in epithelial vacuolization, stratification, and, later, necrosis (Fig. 3.31).

Placenta Following Intrauterine Fetal Death

Following the death of the fetus in utero, the fetal circulation ceases. However, the placenta survives, as maternal circulation within the intervillous space continues. Characteristic changes take place in the placenta postmortem and it is important to recognize them so as not to implicate them in fetal demise.

Macroscopically, the placenta may appear normal if fetal death has been recent but will more likely be pale and feel firm if the fetus has been dead for a while. Obvious causes of fetal demise, such as a massive infarction, retroplacental hematoma, or a large chorangioma, may be found occasionally.

The histological changes may vary depending on the interval between fetal death and delivery of the placenta. No changes may be apparent if the fetus has been dead for less than 6 hours. Intravascular karyorrhexis in the villous vessels is an early postmortem change, being present at between 6 and 48 hours. A striking histological feature is progressive fibromuscular sclerosis of fetal stem arteries, which eventually leads to their obliteration. The villi appear avascular because of capillary collapse and become increasingly fibrotic. If these features are present in 50% or less of the villi, fetal demise most likely occurred between 2 and 14 days before delivery. More marked changes indicate an in utero retention period of over 14 days (Genest 1992). Another prominent feature is an increase in villous syncytial knot formation, thickening of the trophoblast basement membrane, and cytotrophoblast hyperplasia. There may be patchy villous edema with an apparent increase in Hofbauer cells.

Placenta in Fetal Abnormalities

Chromosomal Abnormalities

Placental changes in chromosomal abnormalities are described in Chapter 5.

Inborn Errors of Metabolism

Pathological changes in the placenta have been observed in a number of metabolic storage disorders and in some cases have been the only clue to the diagnosis. Involvement of the various cell types in the placenta is listed in Table 3.1 (Lake 1995).

Electron microscopy on one or two villi from chorionic villus sampling to provide expedient antenatal diagnosis has been used. Ultrastructural evidence of accumulation of metabolites was found in specimens from pregnancies complicated by Niemann–Pick, Hurler's, Pompe's, and sialic acid storage diseases at as early as 10 weeks' gestation.

Fetal Hydrops and Nonimmune Hydrops

A range of findings may be found in placentas from maternofetal rhesus incompatibility or isoimmunization due to ABO or anti-Kell antibodies. Grossly, the placenta may appear normal or be of normal size and weight but show pallor or be bulky, heavy, edematous, and pale. Intervillous thrombosis appears to be a frequent finding. Histologically, the placenta may be normal but may show characteristic changes, particularly in severe maternofetal rhesus disease. There is usually

Table 3.1. Involvement of various cell types revealed by light and electron microscopy (Lake 1995)

Disease	Trophoblast	Fibroblast	Endothelial cell
Sialic acid storage	+ syn and cyto	+	+
GM_1 gangliosidosis	+ syn and cyto	+	+
β-glucuronidase deficiency	+ (patchy)	+	+
Neuramidase deficiency	+ syn and cyto	+	+
I-cell disease	+ syn	+	+
Mucopolysaccharidosis I and II		–	++
Pompe's disease	+ (cyto)	+	+
Aspartylglucosaminuria	+ (cyto, but rare)	+	+
Wolman's disease	–	+ (macrophages)	+
Niemann-Pick A	+ (patchy)	+ (macrophages)	?
Niemann-Pick C	–	–	–
Tay-Sachs disease	+ (syn)	–	–
Mucolipidosis	–	–	+

+, present; –, absent; syn, syncytiotrophoblast; cyto, cytotrophoblast.

villous immaturity, and the stroma may be edematous. Nucleated red blood cells are aggregated in the fetal vessels cursorily resembling extramedullary hematopoiesis. With the development of antenatal diagnosis and treatment, many of the changes described are often not seen.

With nonimmune hydrops, the placenta is usually hydropic and, histologically, resembles that seen in immune hydrops (Fig. 3.32).

Fetal Hypoxia

Three vexed issues arise in relation to fetal hypoxia: whether there is evidence of fetal hypoxia, the duration of the lesion, and whether the lesion is causal. An excess of nucleated fetal red blood cells in the fetal vessels of the placenta in the absence of other causes of increased hematopoietic activity is now accepted as a marker of fetal hypoxia (Fox 1997; Benirschke and Kaufmann 2000). However, nucleated fetal red blood cells are present in quite a high proportion of placentas from term pregnancies but rarely more than 1 per 1000 erythrocytes and their detection may be insensitive (Fox 1967). Unfortunately, the presence of nucleated fetal red blood cells does not provide any reliable information about the timing of the fetal hypoxia. The response time for erythropoietin production and the type and amount of response to acute or chronic hypoxic states are all unknown, while release of fetal red blood cells sequestrated in the liver or spleen also hamper any attempt at estimating the time frame of fetal hypoxia.

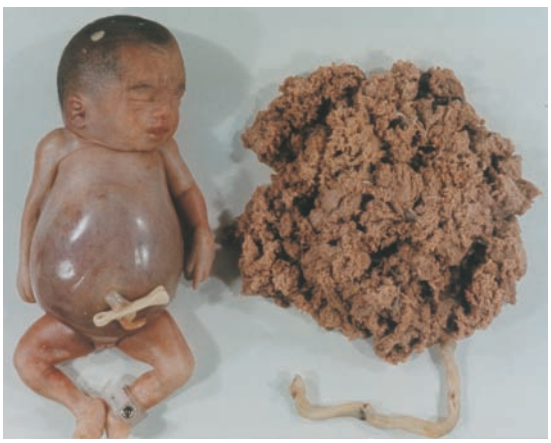

Figure 3.32. Hydropic fetus and bulky pale placenta in thalassemia major. (Courtesy of Prof. W.B. Robertson.)

Meconium staining of the placenta and membranes (see above) is now no longer acceptable as a marker for fetal hypoxia. Fetal hypoxia commonly occurs in association with uteroplacental ischemia due to defective placentation, and the histological changes associated with it may be detectable in the placenta.

Anencephaly

The placenta is usually normal in anencephaly but there may be delay in villous maturation.

Beckwith–Wiedemann Syndrome

Massive hydrops of the main stem villi has been described in placentas from infants with Beckwith–Wiedemann syndrome, allowing ultrasound diagnosis of the condition antenatally (Lage 1991). The histological features may not be characteristic of the syndrome (Pridmore et al. 1994) and may be mistaken for molar changes (Paradinas et al. 2001). A third of the reported cases of mesenchymal dysplasia were associated with Beckwith-Wiedemann syndrome.

Fetal Tumors

Metastases from fetal tumors, especially neuroblastoma and leukemia, are well documented. Single cases of placental metastases from fetal hepatoblastoma and malignant melanoma have been reported. The placenta appears large, pale, and edematous. Histological examination reveals tumour cells plugging villous capillaries.

Placenta in Sudden Infant Death Syndrome

A careful case-controlled study has not found any predictive pathology in placental examination for sudden infant death syndrome when birth weight and gestational age were controlled (Denmead et al. 1987). A morphometric analysis to estimate the efficiency of oxygen transfer across the maternofetal interface found that some components, such as volume, surface area, and fetal capillary surface area, may be reduced in sudden infant death syndrome (SIDS) placentas, but the factors causing the reduction may be associated with the infant being small for gestational age rather than being SIDS-specific (Ansari et al. 2004).

Placenta in Maternal Disorders

Preeclampsia and Eclampsia

Preeclampsia is a disorder characterized by maternal hypertension, proteinuria, and edema. In eclampsia, fitting seizures occur, although women do not necessarily have to go through a prodromic preeclamptic phase. Both syndromes are important clinically because of their association with high perinatal mortality and intrauterine fetal growth restriction and maternal morbidity and mortality.

Placentas from women with preeclampsia are usually smaller than those from uncomplicated pregnancies and contain numerous infarcts; retroplacental hematomas are common. Cytotrophoblast proliferation, thickening of the basement membrane, and an excess of syncytial knots are characteristic of placentas associated with preeclampsia. The villous pattern may appear inappropriately mature for gestation. Accelerated villous maturation has been advocated as a feature in preeclampsia, but observer reliability in assessing villous maturity is problematical. Villi may be hypovascular because of obliterative endarteritis of fetal stem arteries. Fibrinoid necrosis of villi is often seen. These findings are not specific and are all associated with poor uteroplacental blood flow.

There is a defect in the normal invasion of the spiral arteries by the two waves of endovascular cytotrophoblast leading to absence of physiological vascular changes in the myometrial segments of all spiral (uteroplacental) arteries and in the decidual segments of many of these arteries (Fig. 3.33) (Khong et al. 1986). The absence of physiological changes may be incomplete, affecting only part of the circumference of the spiral artery or branches of the same. Where the spiral arteries have not undergone physiological changes, the

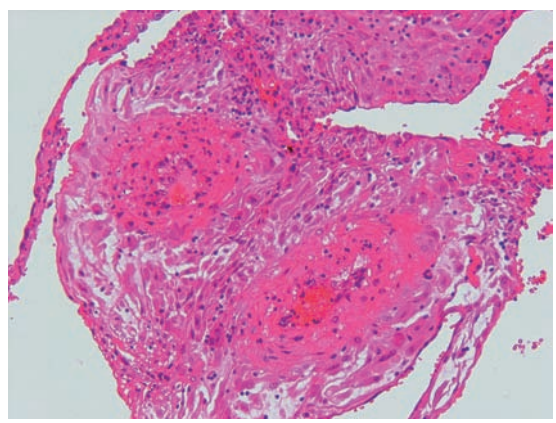

Figure 3.33. Absence of physiological vascular changes in spiral arteries.

undisturbed vascular anatomy would render them responsive to vasomotor influences. A further vascular abnormality in preeclampsia and eclampsia is acute atherosis which is defined as fibrinoid necrosis of the smooth muscle arterial wall accompanied by lipid-laden macrophages and a perivascular round cell infiltrate (Fig. 3.22) (Khong et al. 1987; Labarrere 1988; Khong 1991). Absence of physiological changes and acute atherosis are not specific to preeclampsia and eclampsia, and have been described also in a number of cases of growth-restricted or small-for-dates infants in normotensive pregnancies (Khong et al. 1986). En-face blocks of the basal plate often yield more decidual spiral arteries for assessing the maternal vasculature than conventional sagittal placental blocks (Khong and Chambers 1992). Intraluminal endovascular trophoblast, which is not seen in the third trimester in normal pregnancy, since endovascular trophoblast migration is confined to the first half of pregnancy, is sometimes seen in decidual arteries (Khong et al. 1986; Khong et al. 1992; Kos et al. 2005) (Fig. 3.34). An excessive proliferation of extravillous trophoblast in the implantation site has been described (Redline and Patterson 1995).

These vascular abnormalities explain the reduction in uteroplacental blood flow, and a correlation between the morphological changes in the spiral arteries and uteroplacental blood flow as measured by Doppler uterine artery flow velocity waveforms has been found (Lin et al. 1995).

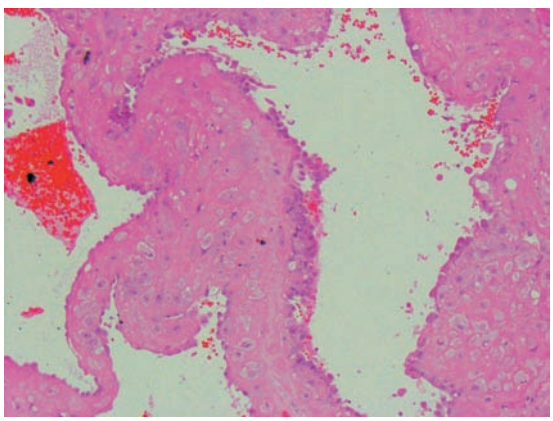

Figure 3.34. Endovascular trophoblast, adhering to the endothelium, within the lumen of a uteroplacental artery from a placenta of a woman with preeclampsia in the third trimester.

Because the number of physiologically converted decidual segments of spiral arteries varies from case to case, the degree of nonconversion may be a factor in determining the volume of blood flow into the intervillous space and, together with the maternal blood pressure driving the blood flow, may explain why some babies in preeclamptic pregnancies are of normal birth weight and why placental infarction is not a uniform finding in preeclampsia.

Diagnostic criteria for identifying maternal vascular underperfusion revealed that several lesions, assessed systematically individually and together, were reproducible. These were increased syncytial knots, distal villous hypoplasia and extent of intervillous fibrin, villous agglutination, acute atherosis, mural hypertrophy of membrane arterioles, basal plate arteries without physiological changes, increased placental site giant cells, and immature extravillous trophoblast (Redline et al. 2004).

Essential Hypertension

In pregnancies in which there is antecedent hypertension, the spiral arteries in the placental bed show hyperplastic arteriosclerosis but uteroplacental arteries are developed in the normal way. In the absence of other complications essential hypertension does not seem to have an adverse effect on placental and fetal growth, and, because of the increased driving blood pressure, may actually result in a baby with a birth weight higher than from a normotensive pregnancy. However, in women with established hypertension who develop superimposed preeclampsia, a defective maternal vascular response to placentation also exists and this is complicated by arteriosclerosis, the consequence of the preexisting hypertension, on which acute atherosis may be superimposed.

Diabetes Mellitus

The many studies of placentas in diabetes mellitus have produced contradictory and confusing results owing to the failure to account for confounding variables such as severity of disease, degree of control of hyperglycemia, premature delivery and other related abnormalities such as preeclampsia.

Reassessment of the claim that placentas from diabetics are, on average, heavier than those from nondiabetic mothers has not been confirmed on a series of well-controlled diabetics, but a relation with maternal glycosylated hemoglobin levels is noted (Clarson et al. 1989). The placenta may be edematous. Umbilical cord edema is frequent, and the incidence of single umbilical artery is increased. Although fetal artery thrombosis is common, there is no increase in infarction.

The range of histological abnormality in placentas from diabetic women may be related to inadequate sampling. Groups of immature villi may be interspersed with areas of accelerated maturity, although 40% of placental villi were of appropriate maturity. Villous edema with prominent stromal Hofbauer cells, fibrosis of the villous stroma, villous fibrinoid necrosis and increase in cytotrophoblast and syncytial knots, thickening of the trophoblast basement membrane, proliferative and tortuous fetal capillaries, and proliferative endarteritis of fetal stem arteries have been described in placentas from diabetic pregnancies. None of these findings is specific, and many placentas are histologically normal. Increased numbers of vasculosyncytial membranes and syncytial knots were found in the placentas of diabetics with imperfect metabolic control between 12 and 32 weeks of pregnancy. The maternal blood supply to the placenta in diabetes mellitus is normal (Vogler et al. 2000; Khong 2001; Faye-Peterson et al. 2005), although decidual vascular disease in the form of fibrinoid necrosis, acute atherosis, and thrombosis has been reported in women with diabetes mellitus and increased resistance in Doppler arcuate artery flow velocity waveforms (Barth et al. 1996).

Morphometric study of the placentas of diabetics showed a significant increase in the volume of parenchyma and a decrease in nonparenchyma (Boyd et al. 1986). The villous surface area was significantly increased even after correction for fetal weight. There is increased branching of peripheral villi in the placentas of diabetic mothers. Electron microscopy has confirmed the light microscopic findings of increased cytotrophoblast, focal syncytial necrosis, and thickening of the trophoblast basement membrane, and has shown increased surface density of microvilli.

Intrahepatic Cholestasis of Pregnancy

Intrahepatic cholestasis of pregnancy is rare, but perinatal mortality, probably due to increased preterm delivery, is increased. No specific abnormality was noted but there was villous edema, cytotrophoblastic proliferation, increased syncytial sprout formation, and no histochemical staining for bilirubin (Costoya et al. 1980).

Collagen Vascular Disease

Much of the literature pertaining to collagen vascular diseases, such as scleroderma and systemic lupus erythematosus, is confused by inclusion of confounding complications such as hypertension and growth restriction. Most placentas are histologically normal. Others may show a massive perivillous fibrin deposition or maternal floor infarction and placental infarction (Van Horn et al. 2004), although the degree of placental infarction is often insufficient to account for fetal death. A vasculopathy similar to acute atherosis has been reported in placentas from women with lupus erythematosus, but pregnancies often have confounding complications that may give a similar morphological feature.

Sickle Cell Disease and Other Hematological Disorders

Sickle crises are more common during pregnancy, and spontaneous abortion and perinatal loss may exceed 50%. The reduced oxygen tension in the intervillous space predisposes to infarction, and placental weight may be increased as a nonspecific adaptation to severe maternal anemia. Microscopically, sludging and characteristic sickling of cells in the intervillous space may be seen, even in the sickle cell trait.

Routine histochemical staining revealed iron deposits at the trophoblastic basement membrane in a term placenta from a woman with homozygous β-thalassemia, although successful pregnancy in this condition is rare (Birkenfeld et al. 1989).

Placentas from pregnancies complicated by severe maternal anemia appear larger, the weight of the placenta being inversely related to the maternal hemoglobin level (Godfrey et al. 1991).

A subsequent study, admittedly with smaller sample size, found no significant association between placental/birth weight ratio and first antenatal hemoglobin concentration (Perry et al. 1995). In women developing anemia in the third trimester, the placental weight was not different from that in nonanemic women, but the placental weight was increased relative to the fetal weight (Lao and Tam 2000).

Maternal hyperhomocystinemia is associated with intrauterine growth restriction, placental abruption, early-onset preeclampsia, and early pregnancy loss. The placentas may show an increased incidence of retroplacental hemorrhage, associated with the abruption, and infarction (Goddijn-Wessel et al. 1996). Findings in the placenta and placental bed were nonspecific and not related to thrombosis per se, although placental weights were reduced (Khong and Hague 1999).

Maternal Malignant Disease

Cancer complicates approximately 0.1% of all pregnancies. Placental metastases from maternal neoplasms have been reviewed (Ackerman and Gilbert-Barness 1997). The commonest tumor to metastasize to the placenta is malignant melanoma. Carcinoma of the breast, bronchus, gastrointestinal tract, cervix, and ovary, and Ewing's sarcoma and leukemias occasionally metastasize to the placenta. Tumor deposits may be visible macroscopically, and histological examination reveals clumps or sheets of tumor cells in the intervillous space. Villous involvement is uncommon, but cases of fetal involvement have been reported. Therefore, the presence of metastasis should alert the clinician to monitor the infant for development of malignant disease.

Cigarette Smoking

The association between maternal cigarette smoking and low birth weight is well established. Placental weights from smokers have been found to be increased, smaller, or not different when compared with those from nonsmoking mothers. There is an increase in placenta previa and abruption among smoking mothers, the latter condition being ascribed to decidual ischemia and necrosis. Atrophic and hypovascular changes in placental villi, as evidence of hypoxia, have been reported in heavy but not in moderate smokers (Mochizuki et al. 1984). Using perfusion-fixed placentas, Burton et al. (1989) showed that there was an increased thickness of the vasculosyncytial membrane separating maternal and fetal circulations and a reduction in capillary volume, but these changes were less severe than those found in studies using immersion-fixed placentas. Villous volume and surface area and villous capillary length are significantly decreased (Larsen et al. 2002).

Placental ischemia may be the result of nicotine-induced constriction of uterine vessels. Cadmium toxicity or accumulation of polycyclic aromatic hydrocarbons may inhibit placental oxidative enzyme systems. Alternatively, nicotine or its metabolites may reduce cytotrophoblast proliferation and subsequent placentation (Zdravkovic et al. 2005).

Cocaine Abuse

No characteristic lesion has been found in placentas from maternal cocaine users.

Intrauterine Growth Restriction

Many, if not all, studies on the placenta in intrauterine growth restriction are actually based on birth-weight charts that indicate that the infant is small for gestational age or small for dates, with the inference that it has suffered intrauterine growth restriction. Thus, some small-for-gestational-age infants may not actually have intrauterine growth restriction, while some babies with intrauterine growth restriction may not be small for gestational age. Further, the lower limit for small for gestational age may be defined differently: less than 3rd, 5th, or 10th centile or less than 2 standard deviations below the mean. Even this approach has drawbacks, including the reliability of the gestational age and influence of ethnic, geographical, and socioeconomic factors on the growth charts.

Many factors are related to poor fetal growth, but when no cause is found it is tempting to attribute the cause of intrauterine growth restriction to *placental insufficiency*, a term that has been deprecated. Gruenwald (1975) argues that as the

placenta is a fetal organ, it cannot be responsible for poor fetal growth, but its growth and development will be affected by factors suppressing or promoting fetal growth. Should the placenta fail in its nutritive function, it is rarely a primary placental problem.

The incidence of placental infarction is increased, but many intrauterine growth restriction placentas show no abnormality apart from being small and may not show any infarcts at all. In all likelihood, the infarcts are merely a marker for an inadequate maternal uteroplacental blood supply. This is in accordance with studies demonstrating inadequate physiological changes in the maternal spiral arteries, implicating a failure of extravillous trophoblast migration during placentation, and the finding of acute atherosis (Khong et al. 1986; Khong 1991). These defects, which are also seen in preeclampsia, can be detected by Doppler flow velocity waveform studies of the uterine arteries (Lin et al. 1995). Others may show changes consistent with uteroplacental ischemia (see Preeclampsia and Eclampsia, above): cytotrophoblast hyperplasia, focal syncytial necrosis, irregular thickening of trophoblastic basement membrane, and the presence of small fetal villous vessels with multilayered basement membranes. There is an increased incidence of villitis in placentas complicated by small-for-dates infants (Russell 1980; Althabe and Labarrere 1985), and the birth weight appears to correlate with the degree of severity of villitis. Doppler flow velocity waveform studies of the umbilical artery have shown that there is an increased resistance in the fetal placental vascular bed (Trudinger et al. 1985), which has been attributed to progressive loss of stem villous vessels (Giles et al. 1985) or to a primary defect in placental angiogenesis (Jackson et al. 1995). Many placentas are histologically normal, however.

Confined placental mosaicism has been found to be associated with some cases of intrauterine growth restriction (Grati et al. 2005) and, also paradoxically, with large-for-gestational-age infants (Wolstenholme et al. 1994). The influence of confined placental mosaicism on extravillous trophoblast migration and the subsequent development of the maternal blood supply is a matter of conjecture at this stage (see Chapter 5). Acute atherosis was reported only in placentas with intrauterine growth restriction associated with confined placental mosaicism but not in placentas with growth-restricted infants without confined placental mosaicism (Wilkins-Haug et al. 1995).

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4
Placental Inflammation

Raymond W. Redline

The placenta and fetus present a substantial challenge to the maternal immune system (Lu et al. 1991). Vigorous local immune responses can potentially activate maternal anti-fetal allograft immunity but a less than adequate local immune response would allow pathogens to enter the placenta and gain access to an immature fetal immune system that is ill-prepared to respond to them. Clearly, the balance between reactivity and suppression is most sorely tested when strong antigens such as microorganisms, alloantigens, novel placental antigens, or maternal autoantigens are expressed in the placental environment and become targets for maternal immunity. Irrespective of their etiology, the resulting immune responses can lead to adverse outcomes such as intrauterine fetal demise, premature delivery, fetal growth restriction, and organ-specific damage to the developing conceptus.

As is the case for all organs, the placenta and implantation site contain populations of resident inflammatory cells that are believed to play crucial roles in placental development and function. The two most prominent (and occasionally misinterpreted) resident populations are large granular lymphocytes in the decidualized endometrium and Hofbauer cells of the villous stroma. The former have been shown to consist primarily of natural killer cells and are believed to play a non-immunologic role in regulating maternal vascular remodeling and trophoblast invasion (Ashkar et al. 2000). They tend to cluster near endometrial glands and maternal vessels without any accompanying tissue damage. The latter are tissue macrophages believed to play a role in villous vascular development and in protection of the fetus from pathogens and other potentially harmful mediators that might inadvertently cross the maternal-fetal trophoblastic barrier (Wood 1983; Anteby et al. 2005). They tend to be monomorphic in appearance and are generally uniformly distributed throughout the villous stroma. They are most prominent in preterm placentas. Notably absent from the uteroplacental arena are neutrophils, eosinophils, and B and T lymphocytes. It is the presence of these latter cells that constitutes pathologic placental inflammation. Acute neutrophil-dominated inflammatory responses (predominantly chorioamnionitis) are observed in approximately 20% of all delivered placentas and are virtually always the result of ascending bacterial infections from the cervicovaginal tract (Kraus et al. 2004). Chronic lymphocytic inflammatory responses occur in 5% to 15% of placentas and are with rare exceptions not associated with documented infection. To what extent these latter reactions represent autoimmune or alloimmune reactions versus the sequela of previous infections or infections by as yet unrecognized pathogens remains an open question at this time.

Acute Chorioamnionitis

Histologic acute chorioamnionitis is defined as a maternal neutrophilic response to bacterial infection with or without an accompanying fetal neutrophilic component (Table 4.1). It should be distinguished from clinical chorioamnionitis, a set of maternal signs and symptoms such as fever,

Table 4.1. Acute chorioamnionitis

Maternal inflammatory response (progression and severity)	
Early	Acute subchorionitis or membranous chorionitis
Middle	Acute chorioamnionitis
Late (days)	Necrotizing chorioamnionitis
Late (weeks)	Subacute (chronic) chorioamnionitis
Severe	Subchorionic microabscesses
Fetal inflammatory response (progression and severity)	
Early	Umbilical phlebitis or chorionic vasculitis
Middle	Umbilical arteritis
Late (days)	Concentric periarteritis
Late (weeks)	Necrotizing funisitis with calcification
Severe	Intense chorionic (or umbilical) vasculitis
Specific patterns	
Acute intervillositis	Maternal bacterial sepsis
Acute intervillositis with intervillous abscesses	*Listeria monocytogenes*
Acute villitis	Fetal bacterial sepsis
Peripheral funisitis with microabscesses	*Candida* spp.

tachycardia, uterine tenderness, and foul-smelling discharge that is neither sensitive nor specific for infection or a histologic inflammatory response. Acute chorioamnionitis is usually the result of infection by organisms present in the cervicovaginal tract. Risk factors for this route of infection include abnormal vaginal flora (e.g., bacterial vaginosis, group B streptococcal colonization), premature cervical dilatation, and rupture of the placental membranes (Blanc 1980). Other less common routes of infection include hematogenous seeding of the placenta (e.g., by organisms causing periodontal disease) and contiguous spread of organisms from adjacent pelvic viscera (e.g., bladder, fallopian tubes, rectum). It has been believed that essentially any organism can cause chorioamnionitis if allowed to enter the placental environment. A recent study demonstrating organisms in many preterm placentas without inflammation challenges this assumption (Steel et al. 2005). In one study, *Ureaplasma urealyticum* was cultured from 65% of placentas with histologic chorioamnionitis, accompanied in over half of cases by other bacteria (Hillier et al. 1988). Evidence suggests that *U. urealyticum* colonization of the fetal respiratory tract increases the incidence of chronic lung disease in premature infants (Lyon 2000). Anaerobic bacteria are cultured from approximately 50% of cases of histologic chorioamnionitis. A smaller percentage of cases are associated with more highly pathogenic organisms (e.g., streptococci, gram-negative bacilli, and staphylococci) that may have an enhanced ability to spread to the fetus. Whether chorioamnionitis caused by these latter organisms confers any additional increased risks in the absence of fetal infection is not known. One study has reported an increased incidence of cerebral palsy in premature infants with *Escherichia coli* infection (Vigneswaran et al. 2004).

The prevalence of histologic acute chorioamnionitis decreases with gestational age from a high of 70% in infants born at less than 30 weeks to approximately 10% at term (Kraus et al. 2004). It has been estimated that approximately 25% of all preterm births are the direct consequence of chorioamnionitis (Guzick and Winn 1985; DiSalvo 1998). Preterm births initially presenting with premature labor and intact membranes represent 45% of all preterm births and have a 14% to 21% incidence of histologic chorioamnionitis. Those presenting with premature rupture of membranes prior to labor represent 30% of cases and have a 26% to 50% incidence of histologic chorioamnionitis. Those delivered because of a direct threat to maternal or fetal well-being (indicated preterm births) constitute the remainder of cases and have a less than 5% incidence of chorioamnionitis. While some cases may develop chorioamnionitis subsequent to their initial presentation, the frequency of positive amniotic fluid cultures at the time of onset of labor and the intensity and pattern of histologic chorioamnionitis support an etiologic role for infection in most cases.

The maternal neutrophilic inflammatory response begins below the chorionic plate with diffuse infiltration of the subchorionic fibrin (acute subchorionitis) and at the decidual-chorionic junction of the membranes following egress from decidual postcapillary venules (acute chorionitis) (Redline et al. 2003). These early (stage 1) infections generally develop over a period of less than 12 hours. Spread into the amnion (stage 2) occurs within 12 to 24 hours. After prolonged infection (>48 hours) neutrophils undergo karyorrhexis, necrotic amnionic epithelial cells become necrotic and are shed into the amnionic fluid, and a bright red band of fibrinoid material accumu-

lates at the amnionic basement membrane. When this pattern occupies over 50% of the sampled membranes a diagnosis of necrotizing chorioamnionitis may be rendered (stage 3). After days to weeks, the acute inflammatory infiltrate wanes and a mixed neutrophilic-histiocytic infiltrate persists in the superficial layers of the chorion. This pattern has been termed subacute (chronic) chorioamnionitis and is correlated with an increased incidence of chronic lung disease (Ohyama et al. 2002). The presence of subchorionic microabscesses at any stage of infection is one manifestation of increased severity and has been associated with an increased risk of fetal sepsis (Keenan et al. 1997).

The fetal neutrophilic inflammatory response to bacterial infection can develop in any gestation over 15 weeks, but is much more frequent in mature fetuses (Redline et al. 2003; Dammann et al. 2004). Fetal responses begin in chorionic vessels or the umbilical vein (fetal stage 1) (Fig. 4.1). They must be accompanied by a maternal neutrophilic response to make a diagnosis of chorioamnionitis. Isolated acute fetal vasculitis is discussed below. Fetal inflammatory responses in the wall of umbilical arteries (fetal stage 2, umbilical arteritis) take longer to develop and are correlated with increased levels of circulating fetal cytokines. Later neutrophils migrate into the stroma of the umbilical cord (Wharton's jelly), where they become aligned in an arc-like configuration around fetal vessels (fetal stage 3). This arc-like configuration, termed by some authors as necrotizing or subnecrotizing funisitis, has been attributed to the deposition of immune complexes composed of amniotic fluid bacterial antigen and maternal immunoglobulins diffusing out from the umbilical vessels (Navarro and Blanc 1974; Hood et al. 1983). After weeks the cells within these arc-like bands can disappear, to be replaced by an eosinophilic precipitate, calcification, and occasionally the development of small capillaries. Severe (grade 2) fetal inflammatory responses, particularly in the large chorionic vessels (intense chorionic vasculitis), have been correlated with an increased risk of cerebral palsy (Redline et al. 1998; Redline and O'Riordan 2000). Fetal inflammatory responses are also sometimes associated with recent nonocclusive mural thrombi, which are additional risk factors for neurodisability. An important caveat is that a fetal inflammatory response is not indicative of fetal infection but rather is a sign of immune activation. Activation is associated with high levels of fetal cytokines (fetal inflammatory response syndrome) and may increase the risk of brain injury and chronic lung disease (Gomez et al. 1998).

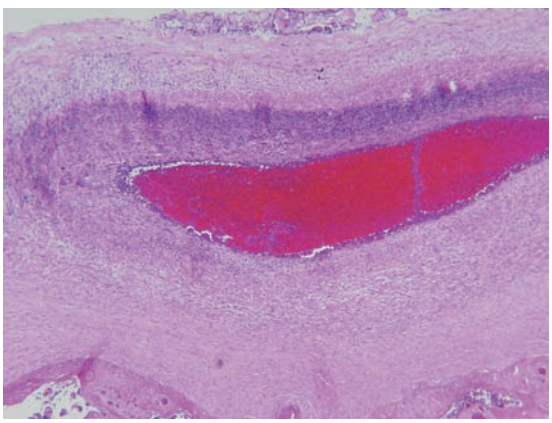

Figure 4.1. Acute chorioamnionitis with a severe fetal inflammatory response (intense chorionic vasculitis). There is confluent fetal neutrophilic infiltration of that portion of the chorionic vessel wall facing the amnionic sac.

Other lesions associated with chorioamnionitis, especially in very premature placentas, include lymphoplasmacytic deciduitis (discussed below) and recent retroplacental hemorrhage ("marginal abruption"), which may develop due to intensive neutrophilic infiltration of marginal uterine veins or changes in uterine geometry that occur after rupture of membranes. Certain specific patterns seen with chorioamnionitis can suggest a particular microorganism. Chorioamnionitis with acute intervillositis and intervillous abscess formation (so-called septic infarcts) is most commonly associated with *Listeria monocytogenes* (Driscoll et al. 1962) (Fig. 4.2). Chorioamnionitis with peripheral umbilical cord abscesses is most seen with *Candida* infection, often in association with rupture of membranes and a foreign body such as an intrauterine device (IUD) or cerclage (Qureshi et al. 1998) (Fig. 4.3). Chorioamnionitis with acute villitis is seen with fetal bacterial sepsis, usually caused by either streptococci or gram-negative enteric bacilli (Langston et al. 1997). Finally, chorioamnionitis with focal acute intervillositis and perivillous fibrin may be seen with maternal

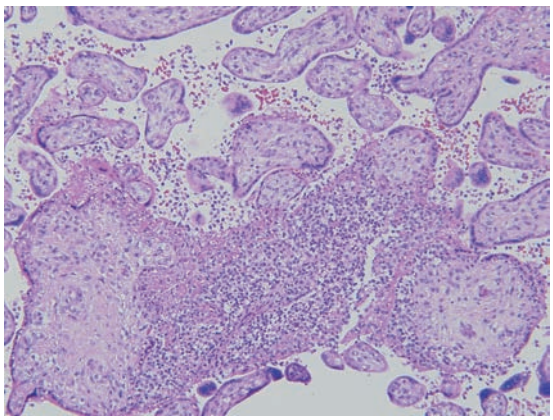

Figure 4.2. Acute intervillositis with intervillous abscesses secondary to *Listeria monocytogenes* infection. Intervillous space and villous stroma are suffused with an acute neutrophilic infiltrate of maternal origin. Gram-positive rods of diphtheroidal morphology may often be demonstrated by tissue gram stain.

sepsis, again usually caused by streptococci or gram-negative enteric bacilli (Bendon et al. 1998).

Adverse outcomes associated with histologic acute chorioamnionitis include all of the complications of prematurity, neurodevelopmental disorders, and chronic lung disease. Intrauterine fetal death is seldom the consequence of chorioamnionitis alone unless there is evidence of overwhelming fetal sepsis. Preterm labor associated with chorioamnionitis has a high recurrence risk. Possible explanations for recurrence include underlying uterocervical structural defects leading to premature cervical dilatation or persisting subacute chronic endometritis caused by organisms in the bacterial vaginosis group.

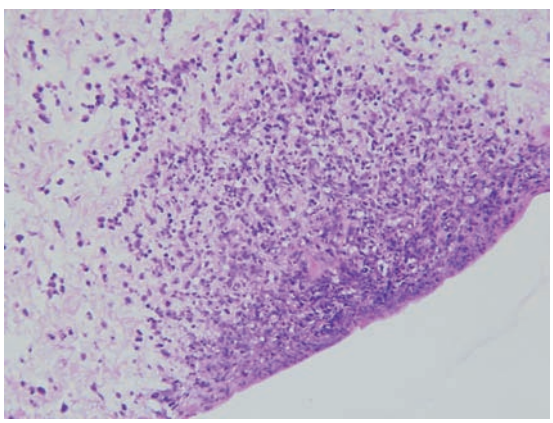

Figure 4.3. Peripheral funisitis with microabscess secondary to placental infection by *Candida* spp. A triangular neutrophilic abscess is seen extending down from the outer epithelial of the umbilical cord. Branching pseudohyphae should be demonstrable by Gomori methenamine silver (GMS) or periodic acid-Schiff (PAS) stain.

Chronic Villitis

Infectious

Chronic villitis is defined as a mixed lymphocytic-histiocytic infiltration of the terminal villous stroma with occasional involvement of stem villi and chorionic plate. Only a small percentage of placentas with these findings are associated with a documented pathogen (Table 4.2). The remaining cases, termed villitis of unknown etiology, are believed to be noninfectious and are discussed below. Chronic villitis caused by placental infection differs from noninfectious villitis in several ways including (1) more common onset in prematurity; (2) more extensive involvement of the villous tree, chorioamnion, umbilical cord, and decidua; (3) histologic features suggesting chronicity such as calcification and fibrosis; and

Table 4.2. Congenital infections with chronic placental inflammation

Chronic villitis, infectious	
Viral	Herpesviruses (CMV, HSV, VZV)
	Rubella virus
	Poxviruses (variola, vaccinia)
Protazoan	*Toxoplasma gondi*
	Trypanosoma cruzi (Chagas disease)
Spirochetal	*Treponema pallidum*
	Borrelia burgdorferi (Lyme disease)
	Intestinal spirochetes
Chronic intervillositis, infectious	
Viral	Measles virus
Parasitic	*Plasmodium* spp. (malaria)
	Schistosoma mansoni
Fungal	*Coccidiodes immitis*
	Cryptococcus neoformans
Chlamydial	*Chlamydia psittaci*
Rickettsial	*Coxiella brunetti* (Q fever)
Bacterial	*Campylobacter fetus*
	Francisella tularensis
	Brucella abortus

CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

(4) evidence of coexisting fetal or maternal infection. The main infections causing chronic villitis in developed countries are cytomegalovirus (CMV), syphilis, and toxoplasmosis. Rarer causes include other herpes viruses [varicella-zoster, herpes simplex, and Epstein-Barr virus (EBV)] and *Trypanosoma cruzi* (Chagas disease) (Altshuler and Russell 1975; Bittencourt 1976; Bittencourt and Garcia 2002). Of primarily historical interest in these countries are cases of rubella virus, smallpox, and vaccinia (smallpox vaccine) villitis. Less agreed upon causes of chronic villitis include enteroviruses, mumps virus, parvovirus, and *Borrelia* spp. (Lyme disease). Other chronic placental infections are limited to syncytiotrophoblast and the intervillous space without villitis. These include congenital infections caused by viruses (measles), fungi (coccidioidomycosis, cryptococcosis), parasites (malaria, schistosomiasis), rickettsia (Q-fever), chlamydia (psittacosis), and unusual bacteria (*Francisella tularensis, Brucella abortus, Campylobacter fetus*) (Renaud et al. 1972; Coid and Fox 1983; Kida et al. 1989; Abramowsky et al. 1991; Friedland et al. 1994; Figueroa et al. 1996; Hyde and Benirschke 1997; Ordi et al. 1998; Ohyama et al. 2001). All of the infections discussed above are believed to spread to the placenta by hematogenous dissemination. Most occur during primary maternal infection in the absence of protective antibodies (Fowler et al. 2003). Placental involvement in patients with chronic or recurrent disease is much less common.

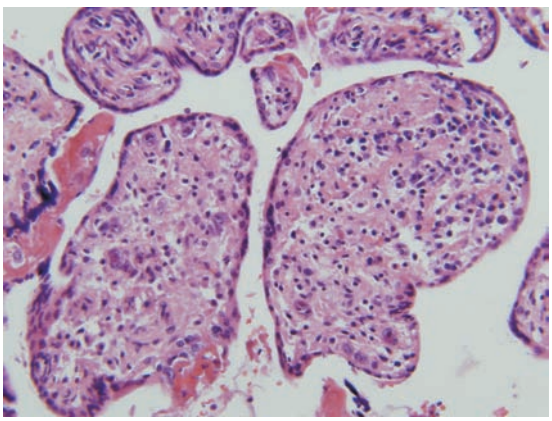

Figure 4.4. Placental cytomegalovirus infection. Terminal villi demonstrate chronic villitis with cytomegalovirus inclusions and stromal plasma cells.

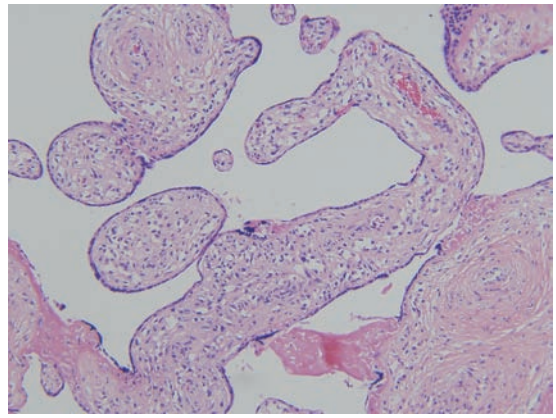

Figure 4.5. Placental syphilis. Terminal and stem villi show a mild diffuse lymphohistiocytic infiltrate with accompanying villous fibrosis. The prominent periarterial sclerosis is typical of syphilis. Spirochetes may sometimes be demonstrated by silver staining of the umbilical cord when inflammation around the umbilical vein is present.

Pathologic findings that allow the pathologist to distinguish between the three most common causes of infectious villitis are as follows. Cytomegalovirus is usually accompanied by a small fibrotic placenta showing focal villous calcification (Mostoufi-Zadeh et al. 1984; (Alba Greco et al. 1992). In cases complicated by congenital hepatitis, hypoproteinemia can lead to hydrops fetalis and a large edematous placenta. The most helpful histologic findings in CMV infection are specific inclusions in the villous stroma, villous stromal plasma cells, and evidence of villous endotheliialitis (hemosiderin deposition, vascular occlusion, and development of avascular villi). The finding of villous stromal plasma cells is virtually diagnostic for CMV (Fig. 4.4). Congenital syphilis is usually associated with a large immature, but not hydropic, placenta showing a predominantly histiocytic diffuse villitis with occasional foci of lymphocytic infiltration (Qureshi et al. 1993). Prominent perivascular fibrosis and occasional perivasculitis surrounding large stem villous arteries can be helpful findings (Fig. 4.5). Almost pathognomonic is the presence in the umbilical cord of necrosis and inflammation extending from the umbilical venous wall into adjacent Wharton's jelly (necrotizing periphlebitis) (Knowles and Frost 1989). Generally, this is the only site in the placenta where spirochetes can be

detected by special stains. The placenta in congenital toxoplasmosis is also generally large, immature, and nonhydropic (Elliott 1970). The villous infiltrate in *Toxoplasma gondii* infection tends to be focal and is often associated with focal villous necrosis or multinucleate histiocytic giant cells. Diffuse chronic deciduitis is prominent. A definitive diagnosis of placental toxoplasmosis is made by demonstration of encysted bradyzoites in the umbilical cord stroma. These pseudocysts are usually apparent in routine hematoxylin and eosin (H&E)-stained sections.

Neonates with congenitally acquired infections involving the placenta often share several common characteristics including cytopenias, pneumonitis, hepatosplenomegaly, and coagulopathy, but also have other specific findings that are organism-dependent (Greenough 1994). Discussion of these latter findings is beyond the scope of this chapter. Adverse outcomes are commonly seen with infectious villitis and may include abortion, stillbirth, prematurity, hydrops fetalis, growth restriction, and long-term sequelae of fetal organ involvement, particularly in the central nervous system.

Idiopathic (Villitis of Unknown Etiology)

The diagnosis of villitis of unknown etiology (VUE) encompasses all cases of chronic villitis not associated with a defined pathogen (Table 4.3). Since the prevalence of infectious villitis in the Western world is approximately 1–4/1000 and the total prevalence of chronic villitis at delivery is between 76/1000 and 136/1000, it is apparent that VUE accounts for over 95% of cases (Kraus et al. 2004). Aside from the lack of detectable placental organisms and the absence of maternal or neonatal signs and symptoms of infection, other factors distinguishing most cases of VUE from infectious villitis include occurrence in term rather than preterm placentas, restriction to distal terminal and stem villi, nonuniform involvement of the villous tree, and the absence of secondary changes such as fibrosis, calcification, endothelial destruction, and edema. Villitis of unknown etiology is not associated with any specific clinical scenario, but patients with autoimmune disorders, obesity, and ovum donation pregnancies are at increased risk (Redline and Abramowsky 1985; Styer et al. 2003; Becroft et al. 2005). Antenatal findings associated with VUE include elevated maternal serum α-fetoprotein levels, nonreassuring fetal monitoring, idiopathic fetal growth restriction at term, and a history of previous pregnancy loss (Salafia et al. 1988; Redline and Patterson 1994).

Table 4.3. Villitis of unknown etiology

Patterns	
Basal	Confined exclusively to basal villi and basal plate
Focal	<10 villi per focus/2–3 foci
Multifocal	<10 villi per focus/>3 foci
Patchy	≥10 villi per focus/<5% of all villi
Diffuse-	≥10 villi per focus/≥5% of all villi
Variant responses	
Granulomatous	With multinucleate histiocytic giant cells
Active	With neutrophils
Associated pathology	
Obliterative fetal vasculopathy	Stem vasculitis/perivasculitis with avascular villi
Chronic intervillositis	Intervillous mononuclear cell infiltrate
Diffuse perivillous fibrin	Recent fibrin surrounding inflamed terminal villi
Decidual plasma cells	Plasma cell infiltrate in decidua basalis
Peripheral funisitis with microabscesses	*Candida* spp.

The pathogenesis of VUE remains unproven. There are two competing theories. First, it has been proposed that an unrecognized common pathogen infects the placenta without spreading to the fetus. Although a recent study has reported viral-like particles by electron microscopy in many cases of VUE, a similar exhaustive search has not been undertaken in placentas without VUE (O'Malley and Gillan 2005). The second theory is that VUE is the result of maternal lymphocytes that cross the syncytiotrophoblastic barrier by chance and enter the fetal connective tissue of the villous stroma. Supporting this theory is the finding by several laboratories that maternal CD8 positive T lymphocytes are the predominant cell in the villous stroma during VUE (Redline and Patterson 1993; Labarrere and Faulk 1995). Maternal T cells in the villous stroma would come in direct contact with fetal antigen-presenting cells bearing foreign class II major histocompatibility antigens inherited from the father. In general, the resulting allograft-type response would be localized, but several scenarios could

promote more generalized, pathologically detectable inflammation. These include an increased precursor frequency of fetal antigen-specific CD8-positive cells in the mother, prior activation of antigen-specific circulating maternal T-lymphocytes, or the presence in the local environment of a co-stimulatory signal that could enhance antigen presentation. This co-stimulatory signal might derive from coexisting genitourinary infection, local inflammation, or increased systemic levels of activating cytokines. Once a significant alloresponse is established, cytokines are capable of upregulating adhesion molecules on syncytiotrophoblast that could facilitate the entry of additional maternal inflammatory cells resulting in more extensive inflammation (Xiao et al. 1997).

The pathologic subclassification of VUE is straightforward (Kraus et al. 2004). In a small subgroup of cases VUE is exclusively basal. Basal VUE is characterized by intense deciduitis with contiguous spread to villi that are embedded in the basal plate (anchoring villi). Unlike the villous stromal infiltrate in other forms of VUE, basal VUE is characterized by a mixed infiltrate of B and T lymphocytes (Fig. 4.6). These cases are sufficiently distinct that they are best considered separately. The remainder of cases may be separated into low- and high-grade VUE based on the number of villi involved in each focus. Cases with exclusively small foci of 10 or fewer villi are low grade and are termed either focal (two or three foci) or multifocal (more than three foci). Cases with more than 10 villi per focus can be separated into two subgroups: patchy (<5% of all villi affected) and diffuse (≥5% of all villi affected). Diffuse VUE is often associated with extensive perivillous fibrin deposition (Fig. 4.7). Another pattern usually confined to high-grade VUE is spread of inflammation to larger stem villi. In these cases chronic perivasculitis or vasculitis can cause fetal vascular occlusion, leading to the development of extensive downstream avascular villi. This pattern has been termed obliterative fetal vasculopathy (Redline et al. 2004) (Fig. 4.8). Other variations in the inflammatory infiltrate associated with VUE include active villitis and intervillositis with numerous neutrophils and histiocytic villitis with granulomatous features. The presence of intervillositis and neutrophils should raise the index of suspicion for infection and consideration of special stains such as the Steiner, Dieterle, or Warthin-Starry stains. Granulomatous inflammation, on the other hand, is commonly seen with VUE and has not been associated with mycobacterial or fungal infection. Special stains for these organisms are not required. Since toxoplasmosis may present with granulomatous villitis (see above), a careful examination of the umbilical cord stroma for pseudocysts and consideration of additional clinical history is recommended.

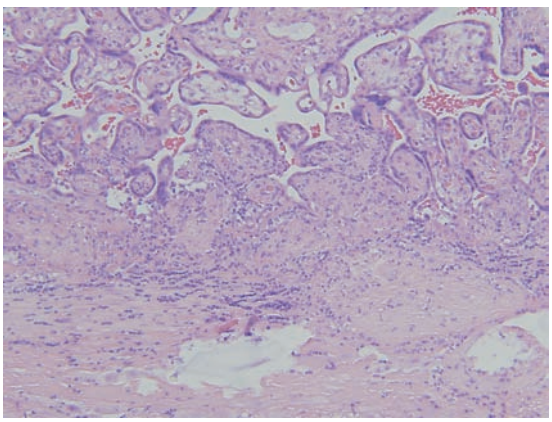

Figure 4.6. Basal chronic villitis (villitis of unknown etiology) with lymphoplasmacytic deciduitis. The decidua basalis is infiltrated by plasma cells and small lymphocytes. The lymphocytic infiltrate extends up into contiguous villi embedded in the basal plate. The remainder of the villi should be free of inflammation.

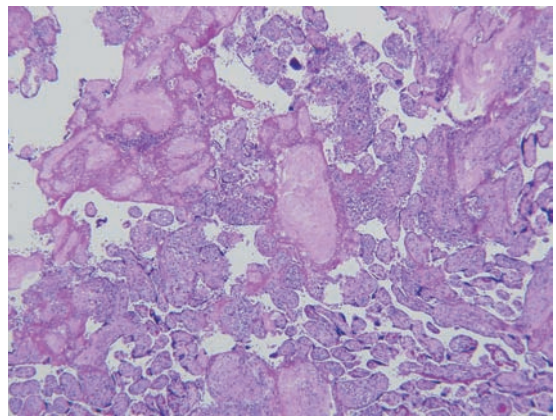

Figure 4.7. Diffuse chronic villitis (villitis of unknown etiology). Terminal villi show marked chronic inflammation of the villous stroma and surrounding intervillous space. Perivillous fibrin is also prominent.

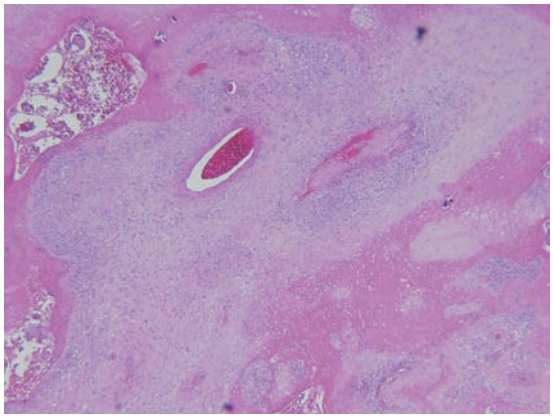

Figure 4.8. Chronic villitis (villitis of unknown etiology) with obliterative fetal vasculopathy. A large stem villus shows a diffuse lymphocytic infiltrate accentuated around stem villous vessels. Several vessels show varying stages of fibromuscular occlusion. One vessel demonstrates a vasculitis with fibrin deposition and a recent adherent thrombus. Downstream terminal villi often show vascular involution and fibrosis in this process.

Basal VUE may be associated with preterm labor and delivery, but rarely with intrauterine growth restriction (IUGR) (Redline 2004). Low-grade VUE, focal or multifocal, is of little clinical consequence. High-grade VUE, either patchy or diffuse, is associated with IUGR. The severity of IUGR generally corresponds to the extent of placental involvement. Diffuse VUE can progress to fetal death and may recur (10–25% of cases) with increasing severity in subsequent cases. In occasional patients diffuse VUE may be a cause of recurrent spontaneous abortion. Villitis of unknown etiology with obliterative fetal vasculopathy is one of a group of lesions affecting large fetal placental vessels that are increased in the placentas of term infants with later neurological impairment (Redline 2005). Other lesions in this group are fetal thrombotic vasculopathy, chorioamnionitis with intense chorionic vasculitis, and meconium associated vascular necrosis.

Other Idiopathic Chronic Inflammatory Lesions

Chronic deciduitis has been defined as either a diffuse band of mononuclear cells extending across the basal plate or localized aggregates of basal plasma cells mixed with other mononuclear cells (Khong et al. 2000). On occasion, chronic deciduitis of either type may also affect the membranes. On rare occasions eosinophils may predominate. It has been proposed that chronic deciduitis is the pathologic correlate of low-grade subacute endometritis caused by mycoplasma or other organisms of low pathogenicity in the bacterial vaginosis group (Goldenberg et al. 2000). In this scenario infections by these organisms could lead to premature labor when the expanding placental membranes come in contact with the colonized endometrium in the late second trimester. The frequent association of chronic deciduitis with acute chorioamnionitis in very low birth weight infants supports this theory. In the absence of treatment, chronic endometritis might cause recurrent premature deliveries. Antibiotic therapy, either empiric or following endometrial biopsy and positive culture, has been recommended by some in this scenario. Villitis of unknown etiology is accompanied by a chronic lymphoplasmacytic deciduitis in approximately 25% to 30% of placentas. In these cases, chronic deciduitis may represent a noninfectious maternal inflammatory response to autoantigens or alloantigens.

Chronic chorioamnionitis occurs in two distinct clinicopathologic scenarios (Gersell et al. 1991; Jacques and Qureshi 1998). The first, discussed above as subacute (chronic) chorioamnionitis, is a protracted form of infectious chorioamnionitis most likely attributable to organisms of low pathogenicity such as mycoplasma or vaginal anaerobes (Ohyama et al. 2002). Occasional neutrophils are mixed with a histiocyte-predominant mononuclear cell infiltrate that is most severe just below the amnion. This pattern is most commonly seen in extremely low birth weight infants and may be an important cause of premature labor. The second process is lymphocyte-predominant inflammation often localized as a band-like infiltrate in the middle to lower chorion. This form of chronic chorioamnionitis is usually accompanied by a coexisting chronic villitis and may be seen with either infection or rare cases of VUE.

Chronic histiocytic intervillositis (massive chronic intervillositis) is defined as diffuse monomorphous infiltration of the intervillous space by CD68-positive macrophages, often accompanied by intervillous fibrin (Valderrama 1992; Doss et

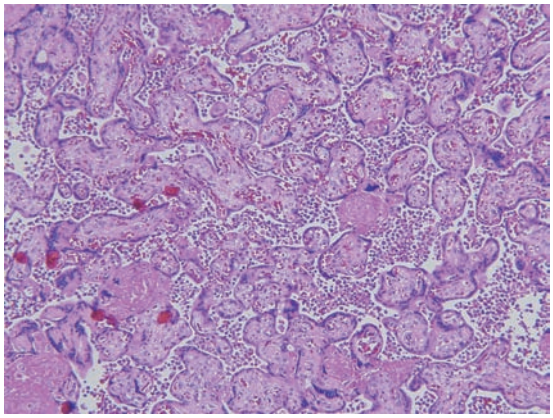

Figure 4.9. Chronic histiocytic intervillositis (idiopathic). The intervillous space is suffused with a monomorphic infiltrate of monocyte-macrophages. All inflammatory cells would stain for CD68. No inflammation should be seen in the villous stroma. Perivillous fibrin is often prominent in this process.

al. 1995; Nijhuis and van Nort 1998; Boyd and Redline 2000) (Fig. 4.9). The presence of a significant number of intervillous lymphocytes or neutrophils or the presence of coexisting chronic villitis would disqualify a case from this category. This uncommon lesion has been associated with recurrent pregnancy losses primarily in, but not restricted to, the first trimester. Patients with this lesion have a high prevalence of associated autoimmune disorders of varying types (Boyd and Redline 2000). Overall perinatal mortality rate is very high and can include abortions, stillbirths, and neonatal deaths. Surviving infants are often growth restricted and electively delivered prematurely due to nonreassuring fetal status. Empiric therapy with heparin, aspirin, intravenous immunoglobulin, progesterone, or corticosteroids has been attempted in selected patients with anecdotal reports of success. Idiopathic chronic histiocytic intervillositis must be distinguished from an unusual pattern of placental malarial infection seen in primiparous patients who first become infected by *Plasmodium falciparum* during pregnancy (Walter et al. 1982; Ordi et al. 1998; Rogerson and Beeson 1999; Mamudo et al. 2000; Rogerson et al. 2003). In addition to their positive exposure history, these latter patients usually have additional histologic findings such as malarial pigment, parasitized maternal red blood cells, and syncytiotrophoblast necrosis (Table 4.2).

Decidual vasculitis/perivasculitis is occasionally seen in and around arterioles of the membranous decidua (Redline 2004). This lesion is most commonly observed in patients with preeclampsia, idiopathic fetal growth restriction, or recurrent spontaneous abortion. It can be seen either alone or in combination with other decidual vasculopathies such as acute atherosis or mural hypertrophy. Whether it is a cause of abnormal maternal vascular remodeling or a clinically insignificant epiphenomenon has not been established.

Isolated fetal vasculitis can affect arteries and veins of the umbilical cord, chorionic plate, or large stem villi. While usually accompanying a coexisting acute chorioamnionitis or chronic villitis, these lesions are occasionally seen alone. Lesions with a neutrophilic or mixed neutrophilic/eosinophilic infiltrate are most commonly associated with prolonged meconium exposure and most commonly occur in the umbilical vein (Burgess and Hutchins 1996). Cases with a lymphocytic or mixed lymphocytic/eosinophilic infiltrate (eosinophil T-cell chorionic vasculitis) are poorly understood and are most commonly observed in chorionic or large stem villous vessels (Fraser and Wright 2002). The relationship of these latter idiopathic lesions to clinical outcome has not been established. If sufficiently severe or extensive, it is not unreasonable to suggest that they might contribute to increased levels of circulating cytokines (fetal inflammatory response syndrome), neonatal coagulopathy, or injury to the central nervous system.

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5
Spontaneous Abortion and the Pathology of Early Pregnancy

T. Yee Khong

Spontaneous Abortion

Definition

The medical profession classifies abortion as induced or spontaneous. The lay public, however, tends to equate the term *abortion* with one that is induced, whether therapeutically, self, or criminal, and to associate the term *miscarriage* with spontaneous abortion (Beard et al. 1985).

Spontaneous abortion is usually defined as the involuntary loss of a conceptus before the fetus has attained viability (World Health Organization 1970). Although this appears to be a simple definition, there are numerous difficulties with it. First, the gestational age at which the fetus attains viability is subject to much debate, especially with the advent of improved medical care of infants who are born prematurely or are of very low birth weight. Second, the legal criteria on viability of the fetus varies among jurisdictions, and definitions of spontaneous abortion have been given as a pregnancy loss at gestational ages varying up to between 20 and 28 weeks (World Health Organization 1970). These differences make it difficult to compare data, and a new definition—"complete loss of conceptuses weighing less than 500g or, when birth weight is unavailable, the corresponding gestation age (22 weeks) or body-length (25 cm crown–heel), irrespective of whether or not there is any sign of life"—is recommended (World Health Organization 1975).

Because spontaneous abortion is so common, and because most spontaneous abortions are followed by a normal pregnancy, the clinical impact of early pregnancy loss is on those couples who experience recurrent abortion. Generally, the term *habitual* or *recurrent abortion* is employed when pregnancies end in abortion on three or more consecutive occasions at about the same period of development. In practice, this definition is often extended to include patients with two consecutive abortions or patients with a total of three early nonconsecutive pregnancy wastages. Some authors distinguish between those recurrent abortions that are preceded by a normal pregnancy, that is, secondary habitual abortions, and those which are not, that is, primary habitual abortions, as their prognosis appears to be different (Goldzieher and Benigno 1958). Another reservation is that the abortions should be with the same partner, as it has been shown that a change in partner may result in successful outcome (Oksenberg et al. 1983).

Missed abortion refers to those pregnancies where the fetus has died in utero, without a defined time span, although older definitions describe a period of at least 2 months. It has been said that missed abortion is a poor descriptive term and that the term "intrauterine death at *x* weeks" is a more accurate way of describing what has happened (Beard et al. 1985).

Incidence

Spontaneous abortion is the most common complication of pregnancy. Assessment of its incidence varies according to the method of ascertainment and definition of the condition used in a given study. Yet, these figures are

important when assessing the safety of antenatal diagnostic techniques and the association of various factors to spontaneous abortion. The rate is approximately 15% of pregnant women but is higher if pregnancies that ended before clinical recognition are also included (Wilcox et al. 1988). Using highly sensitive β-human chorionic gonadotrophin (β-hCG) assays, about 20% of conceptions end before clinical recognition of pregnancy (Wilcox et al. 1988; Wang et al. 2003; Ellish et al. 1996). These occult pregnancies have been defined as "a pregnancy that terminates so soon after implantation that no clinical suspicion exists as to its having existed" (Bloch 1976). For some women, suspicion may be aroused by a delay in menses, but most women would only assume that they are pregnant when they have missed at least two menstrual periods. Thus, many very early pregnancy losses may be dismissed as delayed heavy menstrual periods and not be considered as evidence of pregnancy. Thus, patients having recurrent very early abortions may masquerade as having problems of infertility.

Etiology

General Comments

The etiology of spontaneous abortion is complex, and a large body of literature has accumulated implicating many different factors. In simple terms, these influences range from fetal errors to maternal conditions (Smith 2000). It must be stressed that these factors do not actually explain why and how abortions occur.

Chromosomal Abnormalities

That chromosomal abnormalities are associated with spontaneous abortion is indisputable. Between 50% and 80% of spontaneous abortions are karyotypically abnormal, depending on the maternal age and gestational age at the time of the spontaneous abortion (Lauritsen 1976; Hassold et al. 1980; Hogge et al. 2003). The frequency of abnormal karyotypes from products of conception is no different between recurrent and idiopathic spontaneous abortion (Stern et al. 1996; Hogge et al. 2003). The contribution of chromosomal abnormalities to spontaneous abortion may even be considerably higher as the application of molecular DNA techniques may well reveal a range of chromosomal deletions and rearrangements that are currently not detectable using standard cytogenetic techniques.

Chromosomal abnormalities tend to cause abortion early in pregnancy. Over 50% of successfully karyotyped abortions in the first trimester are aneuploid, whereas only about 10% of spontaneously aborted fetuses after 20 weeks' gestation are aneuploid.

Trisomies account for approximately 50% of spontaneous abortions while monosomy 45XO and triploidy each account for 20% to 25% and tetraploidy the remainder of the spontaneous abortions. Trisomies involving all chromosomes with the exception of chromosome 1 and Y have been described in spontaneous abortion. Between 30% and 50% of such trisomies involve chromosome 16 and virtually all abort spontaneously. About 98% of monosomy 45XO abort but only about 70% of those with trisomies 17–18 or 21–22 abort spontaneously (Edwards et al. 1967). Chromosomal mosaicism can be seen in about 50% of spontaneous abortions (Vorsanova et al. 2005). Survival of an aneuploid fetus may be modified by confined placental mosaicism wherein a proportion of the cytotrophoblastic cells within the placenta are mosaic with a normal chromosomal complement (see p. 110) (Kalousek et al. 1989). Thus, while most fetuses that are trisomic 13 or 18 abort, some may survive, the survival rate being related to the proportion of chromosomally normal "rescue" cytotrophoblastic cells in the placenta (Kalousek 1994). Most chromosomal abnormalities arise de novo, and thus chromosomal analysis of spontaneous abortions is usually unwarranted as it offers little indication of the recurrence risk for that particular chromosomal abnormality. It has been argued, however, that since the risk for spontaneous abortion in a subsequent pregnancy is increased when a normal embryonic karyotype is found in the abortion material, demonstration of a normal embryonic karyotype in a woman with recurrent spontaneous abortion is important for diagnostic evaluation and possible treatment. This karyotypic information would only be available by changing current practice to one of obtaining karyotypes from all pregnancy losses whether or not the patient has a history of recurrent spontaneous

abortion (Stern et al. 1996). Hogge et al. (2003) also used the counterpoint that a subsequent pregnancy is likely to be successful in 75% if the loss is karyotypically abnormal to argue for karyotyping, but they advocated its use after the second loss. A small proportion of abnormal karyotypes result from chromosomal abnormalities in either parent, most commonly a balanced translocation, giving rise to an unbalanced fetal karyotype, and these couples may suffer from recurrent abortions (Simpson et al. 1981). Chromosomal analyses of these parents are warranted.

Congenital Anatomical Abnormalities

Many of the chromosomal abnormalities are associated with gross anatomical abnormalities. There are, however, many gross anatomical abnormalities that are not associated with cytogenetic abnormalities, although this may well change with advances in molecular genetics. Some seemingly minor anatomical abnormalities, such as cleft palate and cleft lip, are overrepresented among abortion compared with liveborn infants. This contrasts with the finding that fetuses with severe malformations, such as complex congenital heart disease, large abdominal wall defects, or neural tube defects generally proceed to term unless terminated following antenatal diagnosis.

Infection

The extent to which infection plays a role in spontaneous abortion is unclear but is likely to be low (Summers 1994). Cohort studies have not shown an association between clinical infection and early pregnancy loss (Simpson et al. 1996). Infection is an insignificant contribution to the cause of recurrent abortion. Systemic maternal illness, for example, lobar pneumonia or pyelonephritis, may cause spontaneous abortion without direct involvement of the fetus or the placenta. The risk of fetal death due to pyrexia, as distinct from infection, may have been overstated (Andersen et al. 2002), and some drugs used in treating severe infections may be teratogenic, thus compounding the effect of pyrexia.

The list of organisms isolated in cases of spontaneous abortion is extensive and those listed in Table 5.1 are by no means exhaustive. However, some organisms have been particularly associated with spontaneous abortion and with fetal death, for example, *Listeria monocytogenes*, *Toxoplasma gondii*, *Campylobacter* species, rubella, cytomegalovirus, parvovirus, herpes simplex, and Coxsackie virus. The role of bacterial vaginosis, defined as a change in the microbial ecosystem in the vagina, in spontaneous abortion is not clear (Hay 2004). It must be remembered, however, that the isolation of organisms from the products of conception by no means establishes causality of spontaneous abortion, particularly when no lesions can be demonstrated in the uterus, placenta, membranes, embryo, or fetus. The products of production may be contaminated by the many organisms in the highly infected birth canal. It is reasonable to argue that hematogenous infection reaching the conceptus via the maternal circulation and leading to fetal death is associated with abortion. It is more difficult when infection is ascending the female genital tract since premature rupture of membranes may precede the infection.

Table 5.1. Infections that may be associated with spontaneous abortion

	Ascending infection	Hematogenous infection
Viruses	Herpes simplex Cytomegalovirus	Cytomegalovirus Rubella Parvovirus Herpes simplex Coxsackie Influenza Hepatitis
Bacteria	*Listeria monocytogenes* *Campylobacter* species *Brucella* *Escherichia coli* *Klebsiella* *Pseudomonas* species *Streptococcus* species	*Listeria monocytogenes* *Campylobacter* species
Other organisms	Mycoplasma Ureaplasma *Candida* species Chlamydia	Toxoplasma Malaria

Maternal Disease

In the evaluation of an early pregnancy loss, the pathologist may be confronted with a history of maternal illness. The association of these factors with spontaneous abortion is not always clear.

Fetal deaths are more common in women with renal disease and are predicted by proteinuria and the degree of renal dysfunction. Women with renal disease and diabetes mellitus and hypertension are at particularly high risk of spontaneous abortion (Holley et al. 1996). Patients who have had Fontan repairs for various types of univentricular heart disease have an increased risk of spontaneous abortion (Canobbio et al. 1996).

Women with celiac disease, whether treated or not, have an increased risk of spontaneous abortion compared with women without celiac disease (Tata et al. 2005). While undiagnosed celiac disease is frequent among pregnant women (about 1 in 80), it did not appear to be associated with an increased risk of spontaneous abortion (Greco et al. 2004) despite an earlier study from the same group examining fewer affected women, which suggested that untreated celiac disease patients had a greater incidence of spontaneous abortion (Martinelli et al. 2000). The pathogenesis may be related to celiac disease–induced malabsorption and deficiency of factors essential for organogenesis, such as iron, folic acid, and vitamin K.

Women who have received a bone marrow transplant incorporating total body irradiation appear to have an increased risk of spontaneous abortion, although study numbers are small (six of 16 pregnancies in 13 women) (Sanders et al. 1996). Pelvic irradiation for childhood cancers was associated with a higher, but not statistically significant, risk of miscarriage (Green et al. 2002).

Hypothyroidism and hyperthyroidism, in themselves, are no longer considered risk factors for spontaneous abortion. However, thyroid autoimmunity is associated with an approximate twofold increased risk of spontaneous abortion in a meta-analysis of case control and longitudinal studies. The association could be due to the increased maternal age of thyroid antibody-positive women or to a general heightened autoimmune state in those women (Prummel and Wiersinga 2004). Luteal-phase defect is not considered to be an important factor; both diagnosis and treatment in recurrent spontaneous abortion are questionable (Coulam and Stern 1994; Dawood 1994). Early pregnancy losses are greater in uncontrolled diabetes mellitus but is no different from the normal population in well-controlled diabetes mellitus (dos Santos Silva et al. 2005).

Cervical incompetence is generally thought to be an important factor in midtrimester abortion but there is no consensus about its diagnosis or its incidence. The length of the cervix has been shown to be inversely related to the risk of preterm delivery throughout the range of lengths, supporting the concept of the incompetent cervix and its possible cause in some midtrimester abortions (Althuisius and Dekker 2005). It is likely that case determination bias and uncontrolled studies have overstated the claim that leiomyomas, particularly submucosal leiomyomas, are associated with an increased rate of spontaneous abortion. Other uterine pathology, such as intrauterine adhesions, has purportedly been implicated in recurrent abortions also by a failure to provide an adequate implantation site. Arteriovenous malformations in the uterus have also been associated with spontaneous abortions. The association of developmental abnormalities and uterine pathology on the one hand and spontaneous abortions on the other is not clear-cut. The majority of women with recognized uterine anomalies and pathology will have successful pregnancies. In addition, in all likelihood, there are many women with unrecognized anomalies or pathology who do not undergo medical scrutiny because they do not have symptoms referable to the abnormality. The association between uterine malformations or pathology and spontaneous abortion is weak and based largely on selective case reporting. The association with early pregnancy wastage was strongest for septate uteri, particularly when the placenta was implanted on the septum. The frequency of uterine anomalies in women with recurrent spontaneous abortions did not appear to be significantly different from women at low risk and without recurrent abortions; however, uterine distortion appeared to be more severe in women with recurrent abortions (Salim et al. 2003).

Reproductive outcome in women with systemic lupus erythematosus is poor with spontaneous abortion and stillbirth. High lupus activity, as determined by the physician, is associated with a threefold increase in early pregnancy loss (Clowse et al. 2005). It is now recognized that the pregnancy loss is associated with the presence of the antiphospholipid antibodies, anticardiolipin

antibody, and lupus anticoagulant, and may be present in women without systemic lupus erythematosus. In this obstetric lupus syndrome, women may suffer repeated early pregnancy losses and late pregnancy stillbirths. First trimester loss of embryonic pregnancies appear to be the most common type of abortion in women with antiphospholipid antibodies. Thrombosis and a direct antibody-mediated damage on trophoblast are possible mechanisms, leading to defective implantation and subsequent placentation (Meroni et al. 2004). Decidual vasculopathy and infarction may be seen in the placenta in the obstetric lupus syndrome (Nayar and Lage 1996), although many other cases are complicated by preeclampsia.

Maternal thrombophilia is associated with spontaneous abortion but the extent of the risks varies among individual studies (Kujovich 2004). The thrombophilias include gene mutations in factor V, prothrombin, methylenetetrahydrofolate reductase (MTHFR), methylenetetrahydrofolate dehydrogenase (MTHFD), plasminogen activator inhibitor-1 (PAI-1) and platelet antigen 2, as well as antithrombin deficiency, protein C deficiency, protein S deficiency, activated protein C resistance, and essential thrombocythemia.

Occupational and Environmental

The association between spontaneous abortion and occupational or environmental factors is based mainly on epidemiological and statistical data. The epidemiological data often are compiled from self-reported patient recall, which may be subject to bias and error, particularly when a single retrospective survey method is used (Farrow et al. 1996). Methodological weaknesses in the studies are inaccurate data on exposure or determination of very early subclinical pregnancy losses.

Exposure to ethylene oxide, a gas used in dental surgeries to sterilize equipment, may increase the risk of spontaneous abortion (Rowland et al. 1996). Women exposed to ethylene glycol ethers, used as organic solvents in industry, had increased risk of spontaneous abortion (Correa et al. 1996). Exposure to toluene, xylene, or formalin, in pathology or histology laboratory settings, was associated with spontaneous abortion (Taskinen et al. 1994). However, a meta-analysis did not find a correlation between paternal exposure to organic solvent and spontaneous abortion (Logman et al. 2005).

The incidence of spontaneous abortion in 689 pregnancies exposed to tricyclic and nontricyclic antidepressants was within the normal range (McElhatton et al. 1996). Therapeutic doses of antimalarials (Phillips-Howard and Wood 1996) or quinolone (Schaefer et al. 1996) do not increase the risk of abortion. Despite variability between studies, particularly in terms of adjusting for confounding factors, a meta-analysis of cohort or case control studies suggested a small but clinically significant detrimental effect of female smoking on spontaneous abortion risk (Hughes and Brennan 1996). Cigarette smoking, by either parent, appeared to be correlated positively with chromosomally normal spontaneous abortions (Kline et al. 1995; Venners et al. 2004). Although most epidemiological studies observe an association between caffeine consumption and spontaneous abortion, the evidence is equivocal because of study designs and biases (Signorello and McLaughlin 2004). Women who were abused domestically had a higher incidence of spontaneous abortion than nonabused women, but there were also confounding factors such as cigarette smoking and taking prescription drugs and antidepressants (Webster et al. 1996). Both paternal and maternal alcohol intake is associated with a greater than twofold increased risk of fetal loss, as measured by urinary β-hCG, but was statistically significant only when consuming 10 or more drinks per week; consumption in the week of conception increased the risk (Henriksen et al. 2004).

Use of nonsteroidal antiinflammatory drugs (NSAIDs) in the first half of pregnancy is associated with an increased risk of miscarriage, but it is unclear whether the effect is due to the drug or to the conditions necessitating its use (Nielsen et al. 2004).

Paternal Effects

It is possible that paternal age may increase the risk of a spontaneous abortion due to mutations of paternal origin. Pregnancies fathered by a man aged 50 or more years had almost twice the risk of ending in a fetal loss compared with pregnancies with younger fathers after adjustment for

maternal age, reproductive history, and maternal lifestyle during pregnancy but the authors caution that the numbers studied are small (n = 124) (Andersen et al. 2004). Another study found that paternal and maternal age are independent risk factors that are synergistic (de la Rochebrochard and Thonneau 2002).

Pregnancies of the partners of male survivors of childhood cancers are more likely to result in spontaneous abortion, and stillbirths and terminations of pregnancy, than pregnancies of partners of their male siblings (Green et al. 2003).

Immunology

Because the placenta and fetus derive half their complement of antigens from the father and therefore is effectively a semi-allograft to the uterus, it is entirely plausible that immunological factors may play a role in spontaneous abortion. Assisted reproductive technology allows transfer of a totally allogeneic embryo. Evidence adduced thus far is circumstantial. Initially, pregnancy was viewed as tolerance by the placenta to maternal T-cell–mediated responses. Later, recognition of a dichotomy of the T cells in mice led to the elaboration of a Th1/Th2 paradigm. Th1 cytokines were proinflammatory while Th2 cytokines were anti-inflammatory, and the hypothesis was that successful pregnancy was associated with a bias toward a Th2 phenomenon, where Th1 cytokines enhance natural killer (NK) cell activity while Th2 cytokines dampen inflammation. Further elaboration of this paradigm came about with the concept of Th3 cytokines, such as transforming growth factor-β, which regulates balance between Th1 and Th2.

This concept of Th1/Th2/Th3 has been challenged. Most of the studies have implied a steady state of these cytokines or were examined after the abortion (Chaouat et al. 2004b). Studies on the antigenicity of trophoblast have focused on histocompatibilty between the woman and her partner. Most studies have not found an increase in major histocompatibility complex antigen sharing in couples and, in particular, no specific histocompatibility complex antigen is associated with recurrent spontaneous abortion. Immunotherapy for recurrent spontaneous abortion by infusion of third-party or partner's leukocytes or intravenous immunoglobulin has not proven to be significantly better than placebo (Chaouat et al. 2004a). Human leukocyte antigen, histocompatibility antigen expression, and complement regulatory protein expression are similar in placental villi from sporadic abortion, recurrent abortion, and terminations of normal pregnancies (Hill et al. 1995). The role of NK cells and cytokines in abortion is unresolved (Moffett et al. 2004). Recognition of cytokine gene polymorphisms may yet uphold some aspects of the Th1/Th2/Th3 concept, but differing associations have been reported already (Prigoshin et al. 2004).

Pathology of Spontaneous Abortion

Classification

Specimens from spontaneous abortion comprise a significant proportion of the workload of most pathology laboratories. Apart from confirming that an intrauterine pregnancy has occurred and excluding gestational trophoblastic disease, pathological examination is performed usually in the belief that the etiology and prognosis for future pregnancy can be determined.

Most abortion tissue reaching the pathologist consists either only of decidua and placental tissue or of decidual tissue with placental site reaction. Fetal tissue may or may not be present, and it is rare to receive an intact fetus or gestational sac. A morphological classification of abortions based on the villous patterns was developed by Rushton (1978, 1984) to try to relate to whether fetal or maternal factors were more likely causes:

- Group I: blighted ova
  - (a) most villi show hydropic change
  - (b) mixed pattern of villi from (a) and (c) with approximately equal proportions
  - (c) most villi show stromal fibrosis and vascular obliteration
- Group II: macerated embryos or fetuses; some villi are hydropic, but most show postmortem change with stromal fibrosis
  - (a) embryo or fetus present
  - (b) embryo or fetus absent
- Group III: fresh embryos or fetuses; villi show no hydropic or postmortem change
  - (a) embryo or fetus present
  - (b) embryo or fetus absent

Figure 5.1. Group I abortus at 8 weeks' gestation with no identifiable fetal parts (blighted ovum).

Placentas from group I are more likely to be from anembryonic pregnancies or in which embryonic or fetal factors may be more important (Fig. 5.1). Pregnancies in groups II and III form the bulk of the midtrimester abortions. With group II pregnancies, it is clear that fetal demise has preceded delivery and it is likely, as in group I, that the cause of pregnancy failure is related to fetal factors (Fig. 5.2). In pregnancies from group III, the fetus was either alive at the time of the abortion or died shortly before the pregnancy terminated, and maternal factors are likely to be more important than in the other two groups (Fig. 5.3).

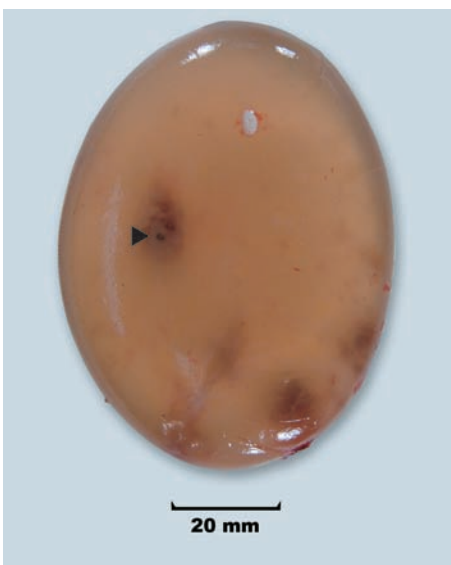

Figure 5.2. Group II abortus at 10 weeks' gestation, showing yolk sac remnant and a macerated fetus with eye structures (arrow) of about 6 weeks' size within the sac, which shows villous degeneration.

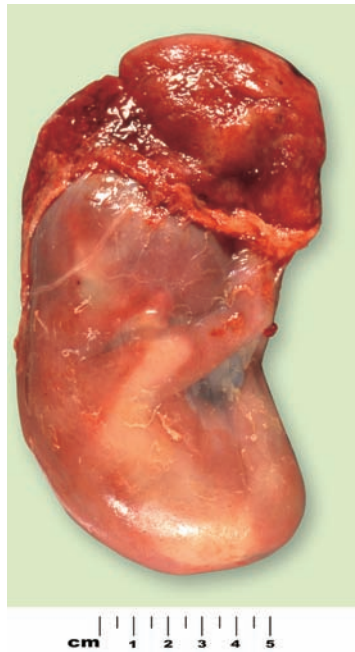

Figure 5.3. Group III abortus at 19 weeks' gestation. A fresh fetus lies within the intact gestation sac.

Although Rushton's classification is commendable for its simplicity, the placental findings were identical in both sporadic and recurrent abortion and could not be correlated with the outcome of future pregnancies (Houwert-de Jong et al. 1990). Furthermore, there is not a reliable correlation between the morphology of chorionic villi and their karyotype (Minguillon et al. 1989; Rehder et al. 1989). With the widespread use of early pregnancy ultrasound, failed pregnancies are also more likely to be induced rather than be expelled spontaneously. Some of the drugs or abortifacients used in inducing labor can result in changes to the placental and or fetal pathology (Lazda and Sams 1995).

Histology

Because a significant proportion of spontaneous abortion is associated with chromosomal abnormality, histological classifications have attempted to correlate histology with a specific chromosomal abnormality or with either aneuploidy or euploidy. The rationale is that karyotyping of all spontaneous abortion material is clearly impractical, but the knowledge of the karyotype is useful for counseling, since couples with a numerical chromosomally abnormal abortion have a better prognosis for a liveborn child in the next pregnancy than couples with chromosomally normal abortion (Stern et al. 1996). Furthermore, some abortions may be due to structural chromosomal abnormalities that are hereditary. Description of histological features associated with a specific chromosomal abnormality after the karyotype is known is less useful than if the histological features had a predictive value for a specific chromosomal abnormality. Features thought to be diagnostic are regularity in size and shape, the presence of trophoblastic inclusions and hyalinization of villous stroma, trophoblastic hyperplasia, and villous vascularity. However, the interobserver variation in judging features associated with karyotypically abnormal abortions can be large, questioning the reliability and applicability of the many studies reporting associations between specific karyotypic abnormality and histological features (van Lijnschoten et al. 1993). Genest et al. (1995) share this view; they found that histopathological differences in spontaneous abortion with identical karyotypes and histological similarities in spontaneous abortion with different karyotypes confounded any attempt to use histopathological assessment of spontaneous abortion as an inexpensive alternative to cytogenetics. One dissenting opinion suggests that using a complicated scoring system for villous and decidual features has a 88% predictive likelihood of chromosomal abnormality and 97% predictive likelihood of chromosomal normality (Salafia et al. 1993). Redline et al. (1999) found inflammatory lesions, such as chronic intervillositis, increased perivillous fibrin deposition with intermediate trophoblast, decidual plasma cells, deciduitis without plasma cells, and chronic villitis, more frequently in karyotypically normal compared with karyotypically abnormal first-trimester spontaneous abortions.

Given the difficulties of observer reliability in identifying karyotypic abnormalities, the failure to distinguish between sporadic and recurrent abortion and the lack of predictive value for future pregnancies, Fox (1993) has argued strongly against continuing to use any form of morphological classification of abortion material. The purposes of examining abortion material would then be to confirm that the patient has been pregnant, to confirm that the pregnancy was intrauterine, to exclude gestational trophoblastic disease, and to attempt to estimate the time of fetal demise.

The systematic examination of fetal specimens obtained from dilation and evacuation procedures can yield clinically important information even though no intact fetus may result from the procedure. It is most useful following prenatal diagnosis of abnormality (see Chapter 6) (Klatt 1995). Excluding karyotyping, the yield from dilation and evacuation procedures following spontaneous abortion is considerably lower, but radiography and gross and microscopic dissection can reveal pathological abnormalities (Kalousek et al. 1990). The reader is referred to this monograph by Kalousek et al., which comprehensively describes and illustrates the principles of early abortion specimen handling and examination.

Pathogenesis of Spontaneous Abortion

When the many factors thought to be responsible for spontaneous abortions are considered critically, it is obvious that they neither actually cause nor account for all the spontaneous abortions. Thus, for example, even though chromosomal abnormalities may be an important factor, there is the occasional fetus with a chromosomal abnormality that is not aborted but that attains viability. It would seem appropriate that explanations are required for the mechanisms of abortions in general, even when they occur in association with the more common causes such as chromosomal and anatomical abnormalities.

Placentation

In many cases of early pregnancy losses, whether sporadic or recurrent, there is inadequate placen-

tation with a failure of extravillous trophoblast to invade the placental bed, in general, and the spiral arteries, in particular (Khong et al. 1987; Hustin et al. 1990) (Fig. 5.4). This extends the spectrum of pregnancy failure in which inadequate placentation is present from preeclampsia and intrauterine growth restriction in the second half of pregnancy to spontaneous abortion in the first half of pregnancy. Control of trophoblast invasion by local immunomodulatory factors, such as uterine NK cells, is debated as a concept unifying immunology and the morphological features, but there is little evidence to support such a role (Dosiou and Giudice 2005). It has been suggested that the failure to invade the spiral arteries leads to premature entry of maternal blood into the intervillous space since these spiral arteries are no longer plugged by intravascular trophoblast. The free passage of maternal blood could then dislodge the trophoblastic shell and result in expulsion of the gestational sac (Harris and Ramsey 1966; Hustin et al. 1990). Absence of or dislodgment of endovascular trophoblast plugs also result in an increase in oxygen tension in the intervillous space and in placental oxidative stress and damage and loss of function (Hempstock et al. 2003). Proponents of this theory point to the absence of intervillous blood flow in early pregnancy (Jauniaux et al. 1994), a view that is disputed by others (Valentin et al. 1996). An alternative view for the role of inadequate placentation in abortion is that abortion occurs as a result of the ischemia induced by failure of conversion of the spiral into uteroplacental arteries, a view that is consistent with abortion in thrombophilic states such as thrombophilia, thrombocythemia, or antiphospholipid antibody syndrome in which there are uteroplacental arterial thrombosis or defective decidual endovascular trophoblast invasion (Van Horn et al. 2004).

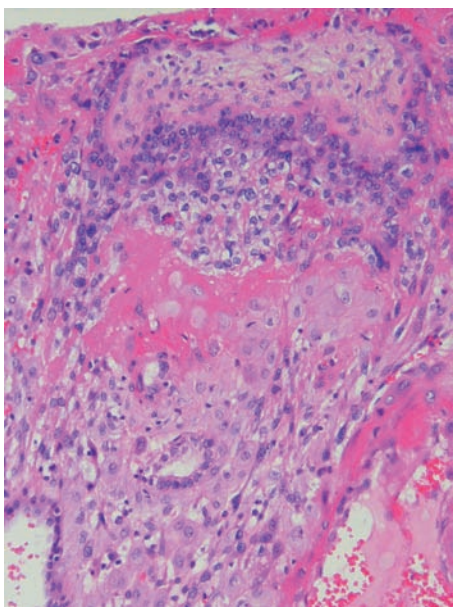

Figure 5.4. Implantation site with anchoring villus. There is paucity of extravillous trophoblast migration into the decidua.

Mosaicism, Uniparental Disomy, and Genomic Imprinting

It is worthwhile considering three recent concepts in cytogenetics: mosaicism, uniparental disomy, and genomic imprinting. Chromosomal mutation may occur at any time between conception and death. A chromosomal mutation in the early stages of development may result in the appearance of an abnormal cell line that may contribute to a significant proportion of the number of cells of the conceptus. The developing conceptus may develop with two or more cell lines with differing chromosome complements, which may be numerical or structural. The presence of two or more cell lines with different karyotypes arising from a common cell line in one individual results in mosaicism. Depending on the timing, site of the origin, and viability of the abnormal cell line, the mosaicism may be generalized, affecting all tissues of the conceptus, or be confined (Table 5.2). The confined mosaicism may be restricted to the embryo or to the placenta (Fig. 5.5).

Confined placental mosaicism results from viable mutations occurring in trophoblast or the extraembryonic progenitor cells of the inner cell mass, the latter contributing to the villous stroma. Confined placental mosaicism is reported in about 2% of viable pregnancies analyzed on chorionic villus sampling at 9 to 12 weeks' gestation. The role of confined placental mosaicism on placental and fetal development is unclear. There is a suggestion that the perinatal outcome is significantly poorer compared with pregnancies without confined placental mosaicism (Johnson et al.

Table 5.2. Confined placental mosaicism*

	Type I	Type II	Type III
Cytotrophoblast	Mosaic or nonmosaic aneuploidy	Normal	Mosaic or nonmosaic aneuploidy
Villous stroma	Normal	Mosaic or nonmosaic aneuploidy	Mosaic or nonmosaic aneuploidy
Fetal tissue	Normal	Normal	Normal

*Adapted from Kalousek (1994)

1990). A large retrospective collaborative study involving 11,775 chorionic villus sampling procedures with 73 cases of confined placental mosaicism failed to demonstrate any marked increase in adverse perinatal outcome (Wolstenholme et al. 1994) and suggested that preferential reporting of positive correlations with adverse outcomes may skew previous reports. It is possible that failure of extravillous trophoblast migration and invasion seen in spontaneous abortion may be secondary to confined placental mosaicism affecting a clone of extravillous trophoblast. The finding of "rescue" diploid cells in confined placental mosaicism in an aneuploid trisomy 13 or 18 fetus that survives (Kalousek 1994) lends support to this hypothesis.

Correction of aneuploidy by early loss of one trisomic chromosome leads to an apparently normal diploid cell line. In one third of such cases, both of the remaining chromosomes are derived from one parent, a phenomenon known as uniparental disomy. Normal development is dependent on the presence of both maternal and paternal chromosomes, although the two parental genomes function unequally due to a process known as genomic imprinting. Imprinting selectively modifies chromosomes during gametogenesis, resulting in parental origin-specific expression of some genes. An example of genomic imprinting involves chromosome 15 where maternal uniparental disomy results in Prader–Willi syndrome, but paternal uniparental disomy

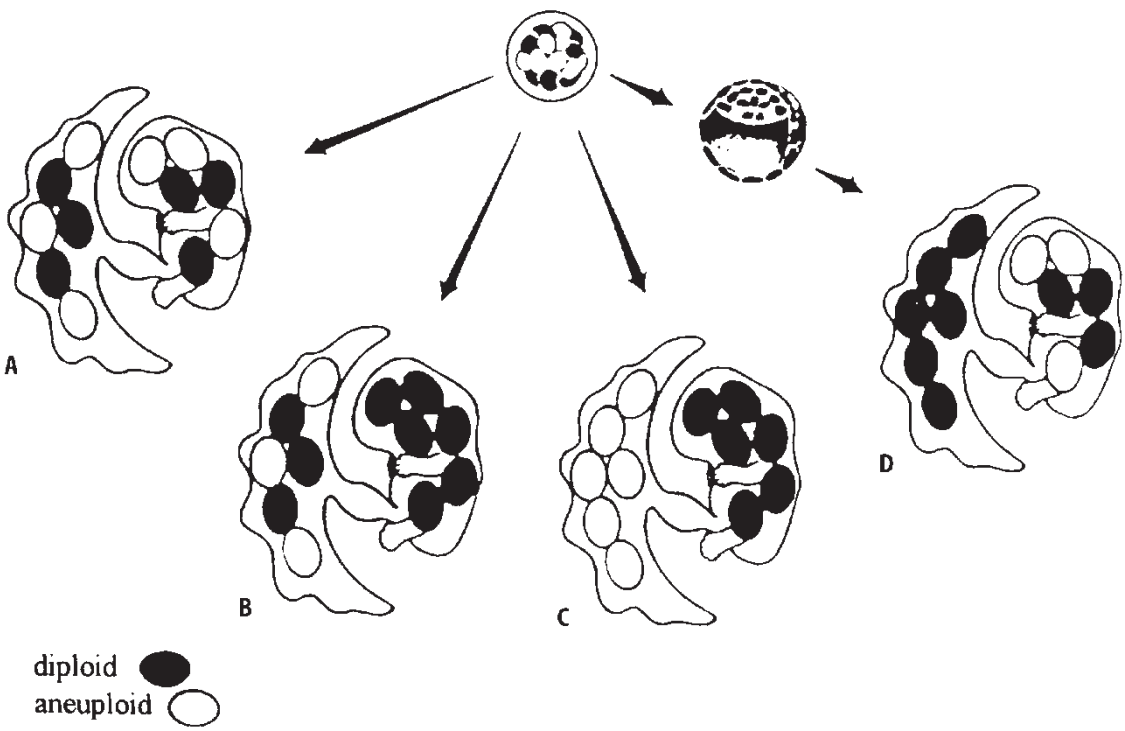

Figure 5.5. Diagram showing different types of mosaicism originating in the preimplantation period. (A) Generalized mosaicism affecting both fetus and placenta. (B) Mosaicism confined to the placenta with normal and abnormal cell lines present. (C) Mosaicism confined to the placenta with only an abnormal cell line present. (D) Mosaicism confined to the embryo. (Courtesy of Dr. D. Kalousek.)

results in Angelman's syndrome. The roles of uniparental disomy and genomic imprinting in adverse perinatal outcome are unclear. Uniparental disomy appears to be infrequently associated with spontaneous abortions beyond 5 weeks' gestation (Fritz et al. 2001; Kondo et al. 2004).

Ectopic Pregnancy

Implantation of the fertilized ovum outside of the body of the uterus is ectopic. The majority of ectopic pregnancies are found within the fallopian tube. Other sites reported are the interstitium of the uterus, ovary, cervix, peritoneal cavity, omentum, spleen, and, increasingly, cesarean section scar. The incidence of ectopic pregnancy has increased from about 0.5% 30 years ago to between 1% and 2% currently (Lehner et al. 2000).

Tubal Ectopic Pregnancy

Over 50% of tubal pregnancies are situated in the ampulla. Approximately 20% occur within the isthmus, 12% are fimbrial, and about 10% are interstitial. Bilateral tubal ectopic pregnancy is rare, with about 200 cases reported in the literature.

It is possible that repeated ectopic pregnancy and increased infertility treatment may explain at least 25% of the increase in the incidence of ectopic pregnancy. It is doubtful, however, if the introduction of more sensitive pregnancy tests or vaginal ultrasound has been contributory (Skjeldestad et al. 1997).

It is difficult to unravel the role of pelvic inflammatory disease in the etiology of tubal ectopic pregnancy (Fox 1995), but nongranulomatous salpingitis is seen in nearly 40% of tubes containing an ectopic pregnancy. Adding to the confusion, *Chlamydia trachomatis*, which is an important cause of pelvic inflammatory disease, may not necessarily be found in the tubes, but chlamydial DNA could sometimes be detected in endometrial and cervical specimens using the polymerase chain reaction (Lan et al. 1995). Risk factors, excluding contraceptive methods, strongly associated with the occurrence of ectopic pregnancy were previous ectopic pregnancy, previous tubal surgery, documented tubal pathology, in utero diethylstilboestrol exposure, infection, and smoking (Ankum et al. 1996; Bouyer et al. 2003). Previous cesarean section does not appear to be an independent risk factor for ectopic pregnancy after adjusting for age, parity, pelvic inflammatory disease, infertility, and smoking (Kendrick et al. 1996). Evaluation of the association between contraceptive methods and the risk of ectopic pregnancy by a meta-analysis of case–control and cohort studies showed that women becoming pregnant after sterilization or while currently using an intrauterine device (IUD) are at an increased risk (Mol et al. 1995). The IUD is associated with an increased risk even after discontinuation of its use (Mol et al. 1995). The literature reports a 5% to 90% incidence of ectopic pregnancy after failed tubal sterilization (Napolitano et al. 1996). A multicenter, prospective cohort study found that a history of tubal sterilization does not rule out the possibility of ectopic pregnancy, even many years after the procedure. The 10-year cumulative probability of ectopic pregnancy for all methods of tubal sterilization was 7.3 per 100 procedures, and the annual rate of ectopic pregnancy was no lower in the 4th to 10th years than in the first 3 years (Peterson et al. 1997). Whether or not there is an association between induced abortion and subsequent ectopic pregnancy is unclear, as many women who have had induced abortions often have several other risk factors for ectopic pregnancy (Skjeldestad and Atrash 1997).

Chromosomal analysis of ectopic pregnancies shows that they are no more likely to have chromosomal abnormalities than in utero conceptuses of comparable gestational age. Ectopic pregnancies were found to have a high incidence of fetal aneuploidy by DNA flow cytometry (Karikoski et al. 1993; Toikkanen et al. 1993). A small study showed that ectopic pregnancies with aneuploid DNA content classified by DNA flow cytometry were less likely to have ruptured (Erel et al. 1996) this finding needs to be confirmed by larger studies, although the sophistication of detection and earlier clinical diagnosis have to be taken into account.

The histology of tubal ectopic pregnancy can be categorized as the aspects of implantation, the placental villi, and the tubal and uterine response

to the tubal pregnancy. Implantation could be plical, fimbrial, mural, or plicomural. Placentation with invasion by extravillous trophoblast and colonization of fallopian branches of the ovarian artery is seen, features akin to those seen in the uterus in an intrauterine pregnancy. The placental villi exhibit the same range of findings as placental villi from intrauterine pregnancies of comparable age, with about one third showing abnormality and two thirds showing changes secondary to fetal death (Fox 1995). Fallopian tubes may show a patchy and inconspicuous decidual reaction. The uterine response to ectopic pregnancy generally shows an Arias–Stella reaction with hypersecretory glands and the endometrium may undergo decidualization. The corpus luteum of pregnancy is contralateral in 30% suggesting ovum transmigration as an etiological factor in tubal ectopic pregnancy (Ramirez et al. 1996).

The sequelae of tubal ectopic pregnancy are abortion without tubal rupture, tubal rupture, or progress to a third trimester unruptured pregnancy. Most tubal ectopics abort spontaneously, particularly those with fimbrial or plical implantation, as there is insufficient supporting tissue for implantation. About 60% of tubal ectopics rupture, largely due to the limited distensibility of the tube to accommodate the developing conceptus but also due to deep trophoblastic infiltration of the thin tubal wall.

Cervical Ectopic Pregnancy

In cervical pregnancy, implantation occurs below the internal os. It occurs in up to 1 in 2500 pregnancies (Marcovici et al. 1994). The criteria for cervical pregnancy are as follows: the cervical crypts must be opposite the placental attachment with juxtaposition of placenta and cervix; the entire placenta must be below the entry point of the uterine vessels or below the site of peritoneal reflection on the anterior or posterior uterine surfaces; and fetal tissue must not be present in the uterine cavity. The diagnosis is frequently not made until the patient is taken for evacuation of a supposed incomplete abortion. Ultrasonography has allowed earlier diagnosis, thereby permitting conservative treatment. The etiology of cervical pregnancy is unclear but is thought to be due to too rapid transport of the fertilized ovum. Transcervical embryo transfer is thought to increase the incidence of this phenomenon in women undergoing in vitro fertilization, but cervical ectopic pregnancy has been described also following gamete/zygote intrafallopian transfer (Qasim et al. 1996). Nonsurgical treatment, such as methotrexate or potassium chloride, has become the treatment of choice and, consequently, pathologists are therefore less likely to encounter curettings from cervical pregnancy. Surgery may cause major hemorrhage or require subsequent hysterectomy.

Ovarian Ectopic Pregnancy

Ovarian pregnancy is rare, occurring in 1 in 7000 to 1 in 40,000 deliveries. Clearly defined criteria must be met to establish the diagnosis: both fallopian tubes must be normal and devoid of any evidence that the initial implantation was tubal; there should be histological evidence of trophoblast; the gestation sac must be connected to the uterus by the ovarian ligament; and the gestation sac should be entirely surrounded by ovary rather than merely being adherent to it.

Some authors have found an increase in incidence of ovarian pregnancies relative to both tubal and term pregnancies but others have not (Gaudoin et al. 1996; Raziel et al. 2004). The etiological factors and pathogenesis are unclear. The pregnancy will not have been suspected in 75%. About one third of patients present in shock and 90% of the pregnancies will have ruptured.

Abdominal Ectopic Pregnancy

Abdominal pregnancy is rare and may be primary or secondary. It is highly probable that most cases are secondary as a consequence of tubal rupture or ovarian pregnancy abortion. The criteria for the diagnosis of a primary abdominal pregnancy are as follows: both tubes and ovaries must be normal without evidence of primary implantation at either site; there must be no evidence of a uteroperitoneal fistula; and the pregnancy must be related principally to the peritoneum. An abdominal pregnancy may implant anywhere within the peritoneal cavity.

Heterotopic Pregnancy

Combined ectopic and intrauterine pregnancies are not uncommon (about 1 in 4000 pregnancies), with most reported concurrent ectopic pregnancies being tubal, although cervical and ovarian ectopics have been described also. The incidence is increasing commensurate with increases in ectopic pregnancy as a result of gamete manipulation (incidence about 1 in 100 to 1 in 500) but heterotopic pregnancies following spontaneous cycles are also increasing, with a 50- to 100-fold increase in risk compared with 20 years ago (Varras et al. 2003; Rabbani and Polson 2005).

Histological Studies

The presence of an implantation site, chorionic villi, or trophoblastic tissue in uterine curettings is conventionally held as definite evidence of an intrauterine pregnancy. Because a tubal ectopic pregnancy could abort into the uterine cavity, chorionic villi do not offer irrevocable proof of an intrauterine pregnancy, for which an implantation site must be seen. The latter, however, does not exclude the possibility of a heterotopic pregnancy. Gross examination of products of conception without accompanying histology can be misleading, with misidentification of fetal parts, placental tissue, and decidua (Spandorfer et al. 1996). Frozen section evaluation of uterine curettings is acceptably accurate for the identification of products of conception on endometrial curettage in the evaluation of suspected ectopic pregnancy (Barak et al. 2005).

Gestational Trophoblastic Disease

Definition

Gestational trophoblastic disease consists of a group of interrelated diseases that can be divided into those with villi and those without villi. The former are the hydatidiform partial or complete moles. The avillous group comprises placental site trophoblastic tumor, choriocarcinoma, and epithelioid trophoblastic tumor (Khong and Ismail 2005).

Partial and Complete Hydatidiform Mole

The incidence of molar pregnancy is difficult to ascertain because of methodological problems in epidemiological studies, including definitions of partial and complete moles and relation of incidences to deliveries or pregnancies. However, it is clear that there is a geographical variation with rates ranging from 1 in 85 in Indonesia to 1 in 2000 in the United States, although the incidence has decreased in those countries with previously high rates (Matsui et al. 2003). A racial difference has been explored. Indians have a higher prevalence of molar pregnancy compared with Chinese in Malaysia and Singapore (Cheah et al. 1993). Moles are commoner in Japanese and local Hawaiians than Chinese and Caucasians in Hawaii. The possibility that the racial differences could be due to dietary and environmental factors was not borne out in two case–control studies from the U.S. and China (Brinton et al. 1989; Berkowitz et al. 1995).

Risk factors for partial molar pregnancy were irregular menstrual cycles, pregnancy histories including only male infants among prior live births and oral contraceptive use greater than 4 years (Berkowitz et al. 1995). The incidence of partial moles was comparable throughout the fertile years but rose to 1.9 times after 40 years of age. Complete moles were commoner between 14 and 25 years of age and after 35 years of age, reaching 4.8 times the average after 40 years of age (Paradinas et al. 1996). By contrast, hydropic abortions were rare before 25 years of age and increased with age to 12 times the average after 40 years (Paradinas et al. 1996).

Clinical Presentation

The most common presenting symptom is vaginal bleeding, occurring in about 85%, but moles can present also as a missed abortion or sonographically detected anembryonic pregnancy. Fewer patients present with the traditional symptoms [excessive uterine size (28%), anemia (5%), pre-eclampsia (1.3%), hyperthyroidism (0%), hyperemesis (8%)] than in the past (Soto-Wright et al. 1995). Early pregnancy ultrasound has resulted in earlier presentation of moles: in a large population-based study, complete moles were evacuated

at about 11.0 weeks' gestation in the period 1985 to 1990 and 9.9 weeks' gestation between 1991 and 2000, while the corresponding times for partial moles were 11.0 weeks and 9.5 weeks, respectively (Matsui et al. 2003).

Genetics and Pathology

Molar pregnancy can be classified on the basis of pathology and genetic origin as partial and complete hydatidiform moles.

Macroscopic examination of the products of conception may reveal occasional cystically dilated villi, especially if the villi are teased out under saline or water. The villi are of variable size and most are of normal caliber. Complete moles consist of an almost uniform mass of grape-like vesicles and no fetal remnants are recognizable. Unlike partial mole, the hydropic change affects all villi, although variably (Fig. 5.6).

Generally, the majority of complete moles are diploid/tetraploid while the majority of partial moles are triploid. The diploid complete moles are androgenetic, that is, genetically derived from the father only. Approximately 85% of complete moles have a 46XX constitution, the remainder having a 46XY karyotype. The extra haploid DNA in triploid partial moles is paternally derived. The vast majority of partial moles are 69XXY but sometimes 69XYY or 69XXX. Not all triploid placentas are partial moles, however; approximately 15% of triploid placentas are maternally derived and are therefore not partial moles morphologically or biologically. A comprehensive study showed occasional exceptions, supported by other studies in the literature (Lage and Bagg 1996; Paradinas et al. 1997). There have been reports of triploid complete moles, diploid/tetraploid partial moles, and tetraploid partial moles, supplanting the simple division of androgenetic diploid complete mole and bipaternally derived triploid partial mole. Be that as it may, hydatidiform moles offer another example of genomic imprinting in humans.

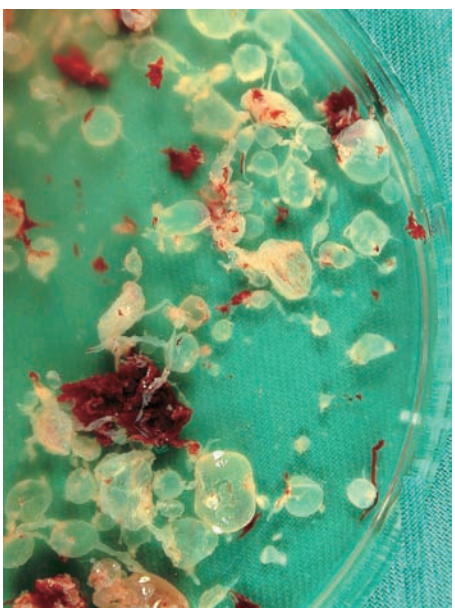

Figure 5.6. Villi teased from a complete hydatidiform mole showing almost universal edema of villi.

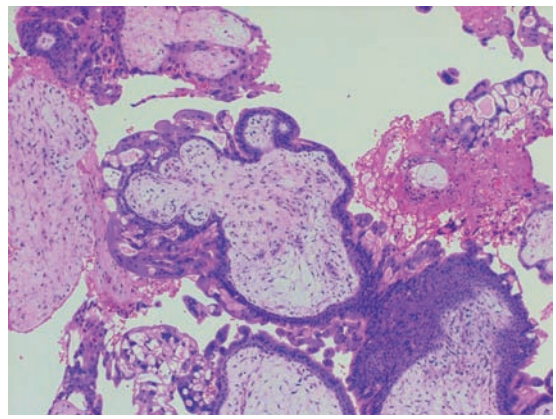

Figure 5.7. An early complete hydatidiform mole showing redundant bulbous projections, cellular stroma, and trophoblastic hyperplasia.

Earlier diagnosis and evacuation of molar pregnancies have necessitated a revision of the diagnostic criteria for hydatidiform moles. Uniform trophoblastic hyperplasia and villous cavitation were classical criteria of complete mole but are now present in less than half the cases. Instead, especially in early moles where hydrops may not be marked, distinguishing criteria are redundant bulbous terminal villi, hypercellular villous stroma, intravillous stromal karyorrhexis, a labyrinthine network of sinuous stromal canaliculi, focal cytotrophoblast and syncytiotrophoblast hyperplasia on both villi and on the undersurface of the chorionic plate, and enlarged hyperchromatic implantation site trophoblast (Keep et al. 1996) (Fig. 5.7). Partial hydatidiform moles have

a range of villi from normal to cystic and the surface trophoblast lining of villi may be attenuated and show minimal proliferation; the villous outlines show geographic, scalloped borders with invaginations of the trophoblast into the stroma to result in trophoblastic inclusions (Fig. 5.8).

In the past, the presence of fetal parts, in the form of embryonic remnants, amnion, fetal vessels, or nucleated red blood cells, was thought to indicate a partial rather than a complete mole. It is now clear that complete moles fulfilling the classic histological criteria, and found to be diploid by DNA flow cytometry, may contain amnion or nucleated red blood cells or have had a history of embryonic development on early ultrasound (Lage and Bagg 1996). The presence of villous stromal karyorrhexis is indicative of complete hydatidiform mole (Szulman and Surti 1978; Paradinas et al. 1996). Implantation site trophoblastic atypia tended to be focal and mild in partial mole and diffuse and marked in complete mole (Montes et al. 1996). Immunohistochemistry to the paternally imprinted and maternally transcribed genes $p57^{KIP2}$ and *IPL/PHLDA2* has been used to distinguish between complete and partial moles (Castrillon et al. 2001; Thaker et al. 2004). Expression is strong in hydropic abortions and in partial moles, which have a maternal genetic contribution, while it is absent or markedly reduced in complete moles, which do not have any maternal genetic makeup.

In practice, the differential diagnosis is between a hydropic abortion and a molar pregnancy since

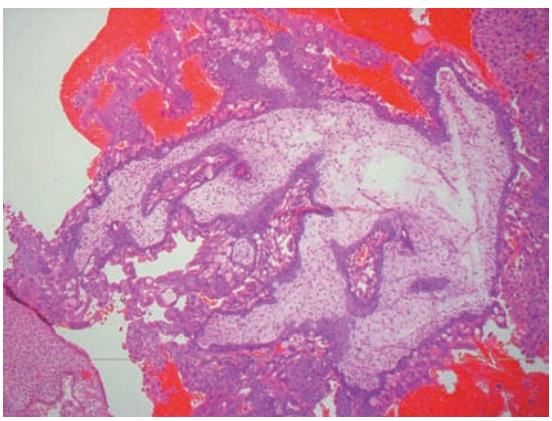

Figure 5.8. A partial hydatidiform mole with scalloping of the villous border and trophoblastic inclusion.

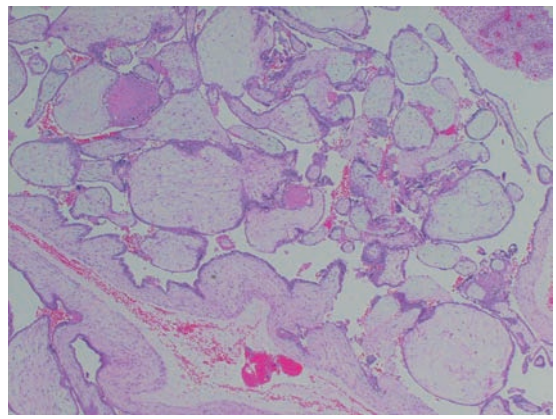

Figure 5.9. Hydropic villi from a miscarriage showing no trophoblastic hyperplasia.

persistent trophoblastic disease can develop from a partial as well as a complete mole. Clinical follow-up of women with a hydatidiform mole using β-hCG remains the same whether the mole is complete or partial (Romaguera et al. 2004). Attention to trophoblastic hyperplasia, villous contours and shape, and intravillous contents, together with immunohistochemistry, should facilitate distinguishing molar pregnancies from hydropic abortion (Fig. 5.9).

β-hCG levels are elevated, more so in complete moles than in partial moles, compared with normal gestations. Other markers, such as inhibin, which is produced by trophoblast, have not supplanted β-hCG assays for monitoring the disease.

Prognosis

Despite the changing presentation of complete moles, there has been no statistically significant change in the development of persistent gestational trophoblastic disease, with about 23% of patients not receiving chemoprophylaxis developing persistent gestational trophoblastic disease (Soto-Wright et al. 1995).

Histological grading, based on trophoblastic hyperplasia and atypia (Hertig and Sheldon 1947), does not correlate significantly with clinical outcome (Genest et al. 1991). Hydatidiform moles that give rise to persistent gestational trophoblastic disease do not have a higher proliferative rate than those that do not (Cheung et al. 1994). Neither

the degree of implantation site trophoblastic atypia (Montes et al. 1996) nor DNA ploidy status (Lage et al. 1992) correlates with the subsequent development of persistent trophoblastic disease.

Twin pregnancies consisting of a complete mole and coexisting fetus have been reported (Bristow et al. 1996), and the coexisting twin can be carried to term with a good outcome, provided that maternal symptoms such as bleeding or pre-eclampsia do not develop.

Invasive Mole, Placental Site Trophoblastic Tumor, Choriocarcinoma, Epithelioid Trophoblastic Tumor

Invasive mole, placental site trophoblastic tumor, choriocarcinoma, and epithelioid trophoblastic tumor present as gynecological rather than as reproductive problems. They are covered only briefly here.

Invasive mole, sometimes referred to as chorioadenoma destruens, refers to the penetration of the myometrium or its vessels by villi from the hydatidiform mole. The vascular invasion may result in extrauterine metastases and hence lead to extensive hemorrhage.

Choriocarcinoma usually follows a gestation and is more likely to follow a hydatidiform molar pregnancy. Choriocarcinoma forms soft hemorrhagic nodular masses and tends to metastasize early. Typically, the tumor is composed of masses of cytotrophoblastic and syncytiotrophoblastic cells, giving rise to a dimorphic picture. β-hCG levels are usually markedly elevated. It is evident that intraplacental choriocarcinomas exist that can serve as an origin for those choriocarcinomas that follow a seemingly normal pregnancy. The alternative term, *choriocarcinoma in situ*, implies absence of metastasis, but that can be ruled out only after an extensive physical and clinical follow-up (Jacques et al. 1998). Intraplacental choriocarcinoma can give rise to both maternal and fetal metastases during pregnancy. Infantile choriocarcinoma as a result of metastasis from the mother is rare. The clinical features of anemia, hemorrhagic liver tumors, and rapid progression to death may highlight an occult maternal choriocarcinoma (Picton et al. 1995).

Placental site trophoblastic tumors present as an enlarging uterus, often with an associated mass. In contrast to choriocarcinoma, there is relatively less hemorrhage, and these tumors are less likely to be preceded by hydatidiform mole. The tumor tends to grow in more confluent and hypercellular mass, with interdigitating growth splitting muscle bundles in the myometrium. Placental site trophoblastic tumors are composed of large mononuclear cytotrophoblastic cells without the dimorphic picture seen in choriocarcinoma and tend to have lower β-hCG levels than choriocarcinoma. Mitotic figures are abundant and aid in distinguishing the lesion from normal implantation site as mitotic figures are rarely seen in normal extravillous trophoblast.

Epithelioid trophoblastic tumour was originally mistaken for an atypical choriocarcinoma that is refractile to conventional chemotherapy. This is a rare tumor, with most following a term pregnancy or spontaneous abortion. Unlike choriocarcinoma, the epithelioid trophoblastic tumor has a monomorphic growth of atypical cytotrophoblastic cells. Occasional syncytiotrophoblastic cells are seen but these are rare. The behavior and appropriate treatment are not clear because of the rarity of the tumor.

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6
Congenital Abnormalities: Prenatal Diagnosis and Screening

Patricia A. Boyd and Jean W. Keeling

Congenital malformations are an important cause of prenatal, perinatal, and infant mortality and morbidity. Three percent of newborns have a single major malformation and 0.7% have multiple major defects. The frequency is much higher prenatally, the majority aborting spontaneously. During the past 50 years, infants with major anomalies have become the focus of increasing and diverse professional expertise and consume a large slice of health budgets in developed countries where their importance as a cause of perinatal mortality has grown as deaths from intrapartum problems have declined and better neonatal care has improved the survival of normally formed low birth weight babies (see Chapter 11). Clinical interest in malformations has been enhanced because sophisticated surgical and anesthetic management makes correction of some major defects possible. This and the recognition of syndromes, their mode of inheritance, and sometimes their etiology requires detailed information from the pathologist in respect to those babies who die, or where termination of pregnancy has been carried out.

There has been rapid expansion of both prenatal diagnostic and screening tests, and in the many different types of disorder that can be detected prenatally. This, and increasing interest in the pathology of the spontaneously aborted fetus, means that pathologists need to be acquainted with malformations in the fetus at an earlier stage of development than previously encountered. They must also recognize the following: that the natural history of congenital malformations is not always known; that malformations are now being defined earlier in gestation; that documentation of abnormalities at different stages is vital; that some disorders may have distinct, gestation-related manifestations; that a major malformation may have different etiologies and thus different recurrence risks; and that coexisting minor dysmorphism may indicate a syndrome diagnosis or a specific etiology. Recognition of the difference between a collection of associated defects that may constitute a syndrome and a chain of events consequent on a single malformation resulting in a sequence of malformations or progressive deformity is important. Detailed observation of normal embryonic and fetal development and of structural abnormalities in the fetus has increased our awareness of the pathogenesis and natural history of many malformations. Our understanding of their etiology has progressed at a much slower rate.

Definitions

An International Working Group (Benirschke et al. 1979; Spranger et al. 1982) addressed the problem of confusion of nomenclature. They defined some essential and widely used terms and concepts relating to malformations. The following definitions are those of Spranger et al. (1982):

- *Developmental field*: a region or part of an embryo that responds as a coordinated unit to embryonic interaction and results in complex or multiple anatomic structures.
- *Malformation*: a morphologic defect of an organ, part of an organ, or larger region of the

body resulting from an intrinsically abnormal developmental process.
- *Disruption*: a morphologic defect of an organ, part of an organ, or a larger region of the body resulting from the extrinsic breakdown of, or an interference with, an originally normal developmental process.
- *Deformation*: an abnormal form, shape, or position of a part of the body caused by mechanical forces.
- *Dysplasia*: an abnormal organization of cells into tissue(s) and its morphologic result(s). In other words: a dysplasia is the process (and the consequence) of dyshistogenesis.
- *Polytopic field defect*: a pattern of (widely separated) anomalies derived from the disturbance of a single developmental field.
- *Sequence*: a pattern of multiple anomalies derived from a single known or presumed prior anomaly or mechanical factor.
- *Syndrome*: a pattern of multiple anomalies thought to be pathogenetically related and not known to represent a single sequence or a polytopic field defect.
- *Association*: a nonrandom occurrence in two or more individuals of multiple anomalies not known to be a polytopic field defect, sequence, or syndrome.

In practice, it may be difficult to distinguish between malformations and disruptions, particularly when exposure to a teratogen occurred early in embryogenesis.

Causes of Congenital Malformations

The causes of malformations can be considered in five broad groups: mutant genes, chromosome anomalies, multifactorial disorders (the result of interaction between genetic predisposition and presumed environmental factors), teratogenic agents, and those of unknown cause. We know that in addition to their adverse effects on embryogenesis, single-gene defects, chromosome anomalies, and environmental teratogens have a range of effects on the developing human organism. These include loss of zygotes resulting in infertility, early miscarriage, fetal growth restriction, alterations of system function, and predisposition to malignant neoplasms. Some of these other effects may also be present in the individual with a structural malformation. Exciting developments in genetics, such as evidence of genes involved in the regulation of development, awareness of microdeletion syndromes, imprinting mechanisms, uniparental disomy (the inheritance of both homologues of a chromosome pair from only one parent), and mitochondrial disorders, have given new insight into the etiology of some of these conditions. For a fuller discussion of single-gene defects, chromosome anomalies, and genetic counseling, see Firth and Hurst (2005), Strachan and Read (2004), Gardner and Sutherland (2004); Rimoin et al. (2002), and Jones (2005). A useful overview of the causes of congenital anomalies and of environmental risk factors is available at www.eurocat.ulster.uk/pubdata.

Despite enormous advances in genetics over the last decade, particularly in gene mapping and the elucidation of specific structural changes within mutant genes, the etiology of around 60% of malformations is unknown. Mutant genes, chromosome anomalies, or known teratogens can be identified in about 6% to 7% each of cases, with a further 20% of malformations falling into the group of multifactorial disorders. Even when an etiological agent is identified, the actual mode of action is usually not understood.

Single-Gene Defects

These disorders are the result of a single mutant gene and are inherited in mendelian fashion. Many individual disorders are rare and others do not result in anatomical defects. However, a very large number of disorders have been identified: OMIM (On-Line Mendelian Inheritance in Man; www.ncbi.nlm.nih.gov/Omim/) lists nearly 2000 entries where a description of a phenotype is available and the molecular basis is known. Single-gene defects underlie approximately 7% of congenital malformations at term, roughly the same proportion as are caused by chromosome abnormalities. Mendelian disorders can be inherited in different ways: autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive.

Autosomal Dominant

Autosomal dominant genes give rise to recognizable effects in heterozygous individuals, usually

with an equal sex distribution. A parent with an autosomal dominant condition has a one in two chance of passing the disorder to each offspring. Some of these disorders may not produce recognizable disease until adult life, for example, Huntington's chorea and adult renal polycystic disease. Others, such as achondroplasia, are recognizable at birth and may be suspected in the late second or third trimester by ultrasound examination with confirmation by appropriate analysis of DNA from fetal sampling. It is clearly important to distinguish this from other types of skeletal dysplasia (see Chapter 29). An increasing number of skeletal dysplasias have been characterized at the molecular level. It is interesting that the number of genetic loci involved is fewer than anticipated, with several examples where phenotypically distinct conditions are allelic variants. Most of these conditions have several different mutations described so that prenatal diagnosis is not straightforward. Achondroplasia is the most common skeletal dysplasia in humans and is unusual in that 98% of cases are due to two common mutations in the fibroblast growth factor receptor type 3 (FGFR3) locus on chromosome 4p. Thanatophoric dysplasia (lethal) is also caused by mutations in FGFR3; Reardon (1996) and Ikegawa (2006) review skeletal dysplasias detectable by DNA analysis.

With an increasing number of single-gene disorders being characterized at the molecular level it is apparent that what may have been considered a single disease clinically may be very heterogeneous in terms of different mutations or loci causing a similar phenotype. Hypertrophic cardiomyopathy (HCM) is an example of this with, to date, several different loci identified on different chromosomes and about 70 individual disease-causing HCM mutations identified so far (Maron 1997). Thus, for many single-gene defects, routine molecular diagnosis is not yet a practical option and remains a research tool.

Some dominant genes can be identified directly and others indirectly using molecular biological techniques, for example, adult polycystic kidney and myotonic dystrophy genes. Myotonic dystrophy is an example of a disease caused by a triplet repeat (CTG) expansion. There is close correlation between the severity of disease and the size of expansion of the repeat sequence in the gene. Small length mutations produce few or no symptoms, but amplification in successive generations can result in increasing disease severity. Congenital myotonic dystrophy (which may be suspected prenatally when polyhydramnios and bilateral club foot are identified on scan and the mother has myopathic symptoms) is always inherited from an affected mother. Prenatal diagnosis is possible for this condition by direct DNA analysis.

When an isolated autosomal dominant disorder occurs and the parents are unaffected, it is likely to be a new mutation and will not recur in siblings. However, the possibility of gonadal mosaicism, reduced penetrance, and variable expression means that there may be a small but real recurrence risk.

Autosomal Recessive

Autosomal recessive genes occur in males and females equally and are only clinically manifest in the homozygous state. Thus, affected individuals have healthy heterozygous parents. Unless an autosomal recessive gene is common in the population (e.g., cystic fibrosis in Western European Caucasians, Tay–Sachs disease in Ashkenazi Jews, β-thalassemia in people originating from Mediterranean countries, North and West Africa, the Middle East, India, Southeast Asia, south Russia, and south China), there is often a history of consanguineous marriage. Because the recurrence risk for affected siblings is one in four, it is important that these conditions be recognized rapidly so that families can be appropriately counseled.

Recessively inherited disorders likely to be encountered by pathologists among perinatal deaths are Meckel's syndrome, infantile polycystic renal disease (Chapter 22), short-ribbed dwarfing syndromes (Chapter 29), cystic fibrosis with meconium ileus (Chapter 18), and Werdnig–Hoffmann syndrome (spinal muscular atrophy type 1) (Chapter 28). The molecular basis of Meckel's syndrome has been characterized at the molecular level in some Finnish families, but has been shown to be heterogeneous with families from outside Finland not linked to the same locus (Salonen and Paavola, 1998)

Some genetic metabolic disorders such as glutaric acidemia type II (see Fig. 6.19) and Zellweger's syndrome (see Chapter 7) exhibit a range of

malformations. These are thought to result from an autoteratogenic effect of the abnormal fetal biochemical milieu due to accumulation of metabolites because of a specific enzyme deficiency.

X-linked Dominant

X-linked dominant disorders typically affect female hetrozygotes mildly and male hemizygotes severely. Affected males are often miscarried. Examples are Rett syndrome (mutation in MECP2) and oral-facial digital syndrome Type 1 (mutation in FMNA filamin A) (Firth and Hurst 2005).

X-Linked Recessive

X-linked recessive genes, carried on the X chromosome are expressed in the hemizygous state. Usually only males are affected, and the disorder is transmitted from healthy female carriers. Examples are Duchenne muscular dystrophy and hemophilia. All daughters of affected males are obligate carriers and all sons are unaffected. The sons of carrier females have a one in two chance of being affected and the daughters a similar chance of carrying the gene. In some of these conditions gene carriers can be identified biochemically or by using DNA techniques. See Emery (1998) for a review of the muscular dystrophies and Abbs (1996) review of the prenatal diagnosis of Duchenne and Becker muscular dystrophy.

The fragile X mental retardation syndrome warrants special mention; since the discovery of its molecular basis, the transmission of the triplet repeat sequence in the FMR1 gene has been studied in families, and the unusual features of inheritance of this syndrome (not straightforward X-linked) are now understood (Gardner and Sutherland 2004). It is the most common form of inherited mental retardation, with approximately 1 in 5000 males carrying the full mutation with intellectual disability. Confirmation of diagnosis has moved from cytogenetics to the molecular laboratory and accurate prenatal diagnosis is now possible, with the exception of predicting the phenotype in females with a full mutation.

Chromosome Abnormalities

Normal human development is dependent on the correct chromosome complement, usually 22 homologous pairs of autosomes and one pair of sex chromosomes. Too much or too little chromosome material usually results from a fault occurring during meiosis. Sixty percent of first trimester abortuses and 5% of stillbirths are chromosomally unbalanced. Karyotyping these groups and any dysmorphic fetus or baby is particularly important.

There is a huge range of chromosome disorders, many of which are beyond the scope of this chapter, but Schinzel (2001) provides a useful catalogue. Commonly encountered abnormalities in the aborted fetus are those involving whole extra haploid sets of chromosomes polyploidy (e.g., triploidy, 69 chromosomes) and aneuploidy, the loss or gain of a whole chromosome (monosomy and trisomy, respectively). Both polyploidy and monosomy (with the exception of a small proportion of monosomy X) are virtually lethal in humans. Structural chromosome abnormalities may involve translocations (exchange of material between chromosomes), deletions, or duplications (loss or addition of chromosome material). Gardner and Sutherland (2004) describe the etiology and mechanisms of chromosome disorders.

Triploidy

Triploidy occurs in approximately 6% of recognized pregnancies. It usually results from an error at fertilization, an ovum being fertilized by two spermatozoa, occasionally by a diploid sperm and less usually from fertilization of a diploid egg. The karyotype is most commonly 69,XXY. In those triploids resulting from a double paternal contribution (diandry), the placenta is abnormal with partial molar change; pregnancy rarely proceeds beyond the first trimester. Half of these pregnancies abort spontaneously, and most of the rest persist as missed abortions. Invasive mole is an infrequent sequel (see www.hmole-chorio.org.uk).

Triploids resulting from fertilization of a diploid egg (digyny) do not have molar change in their placentas, and pregnancy may continue beyond the first trimester. The difference in development of the embryo and of trophoblast growth and function is related to the parental origin of the extra set of chromosomes (Jacobs et al. 1982). This was the first recognized manifestation of genomic imprinting in the human.

Tetraploidy (92 chromosomes) is uncommon. Warburton et al. (1991) found it in 2% of abor-

tuses in their study. Ploidy and sex chromosome composition can be assessed on tissue sections using probes to specific regions on chromosomes and in-situ hybridization techniques (van de Kaa et al. 1991). This can be useful when culture fails or when examining archival material (see also Chapter 5).

Autosomal Trisomy

An additional chromosome is much more commonly seen than is a chromosome loss. Trisomy of most autosomes has been recorded, but the incidence varies enormously. Overall, trisomy of chromosome 16 is the most common (Warburton et al. 1991), but the usual result of this anomaly is spontaneous or missed abortion in the first trimester. About 15% of cases have a disorganized embryo; more advanced embryonic development is present in less than 10% of cases and many of these are mosaics. When liveborn trisomic infants are examined, trisomy 21 (Down syndrome) is the most common followed by trisomy 18 (Edward's syndrome) and trisomy 13 (Patau's syndrome). It is important to remember that even among these karyotypes, miscarriage is the most common outcome. The majority of trisomic pregnancies perish at between 10 and 15 weeks of gestation. Ninety-five percent of Down syndrome, nearly all Edward's syndrome, and 80% of Patau's syndrome are regular trisomies arising by nondisjunction at the first or second meiotic division. A small proportion of cases have a translocation either arising de novo or inherited from a parent who carries a balanced translocation. Mosaicism involving a normal and a trisomic cell line arises either by loss of an abnormal cell line in an abnormal conception by anaphase lag or from postzygotic mitotic nondisjunction in a previously normal conceptus. Such individuals exhibit abnormalities that may be less severe than pure trisomic individuals (see Jones 2005 for details of phenotypes).

Structural Chromosome Abnormality

Structural chromosome rearrangements such as translocations, inversions, and deletions may arise de novo or as a result of a parental chromosome rearrangement. Fusion at or near the centromere of the five acrocentric chromosomes (Robertsonian translocation) is one of the most common balanced structural rearrangements. Simple reciprocal translocations involve exchange of material between two chromosomes. A break occurs in one arm of each, and chromosome material distal to the break swaps positions. Balanced carriers are entirely normal, having no gain or loss of chromosome material, but they are at risk of having chromosomally unbalanced offspring or miscarriages due to malsegregation at meiosis. The extent of the chromosome imbalance depends on the particular translocation.

Unbalanced structural chromosome rearrangements (arising de novo or resulting from an inherited rearrangement) result in deletions (partial monosomy) and duplications (partial trisomy). These often produce structural anatomical abnormalities, which in a few cases (e.g., 4p, Wolf–Hirschhorn syndrome) are sufficiently characteristic to suggest the nature of the chromosome defect. *Microdeletion syndromes* such as Prader–Willi and Angelman's (chromosome 15), 22q deletion syndromes [DiGeorge, velocardiofacial (Shprintzen)], Williams' syndrome (chromosome 7), Miller–Dieker and Smith–Magenis (chromosome 17), are being recognized with increasing frequency as techniques for chromosome examination are improved. See Firth and Hurst (2005) for a description of submicroscopic chromosome abnormalities and the chromosomal phenotype.

If one of these conditions is suspected and no visible deletion found on microscopic examination of a karyotype, DNA probes may identify one. It has become apparent that deletion of chromosome 22q11 is associated with a wide variety of clinical phenotypes (Ryan et al. 1997; Goodman 2003).

Routine Giemsa (G) banded karyotyping is now supplemented by new molecular cytogenetic and noncytogenetic techniques, such as fluorescence in-situ hybridization (FISH), submicroscopic telomere analysis, comparative genomic hybridization (CGH), microarray analysis, and molecular analysis of telomeric sequences, as outlined in Gardner and Sutherland (2004) and Strachan and Read (2004).

Genomic imprinting is the difference in phenotypic expression of a genetic disorder depending on the maternal or paternal origin of the mutation. It may go some way toward explaining observations that apparently contradict simple mendelian inheritance (Donnai and Read 2003).

Examples of imprinting in humans include the different phenotype in triploids, which depends on the parent contributing the extra haploid set, and Prader–Willi and Angelman's syndromes, in which there is microdeletion at 15q11–13 in the majority of cases and uniparental disomy (UPD) in a minority. Phenotype here reflects the parental source of the missing genetic material. Other expressions of genetic imprinting are the clinical observations of difference in severity of inherited disorders such as Beckwith-Wiedemann syndrome (BWS), neurofibromatosis, and Huntington's chorea when inherited through the father or mother (Hall 1990).

Uniparental disomy (UPD) (Engel 1993) occurs when both pairs of homologous chromosomes are inherited from one parent (i.e., both maternal or both paternal). It is caused by a meiotic nondisjunction event, followed by trisomy or "monosomy rescue." Advanced maternal age, structural chromosome abnormalities, and supernumerary marker chromosomes are associated with an increased risk for UPD. There may be no phenotypic effect or one due to trisomy of the placenta or fetus, autosomal recessive disease due to homozygosity, or, for some chromosomes, imprinted gene effects.

In *mitochondrial inheritance* (see Poulton et al. 2003) mitochondria are passed on in the cytoplasm of the egg, so all mitochondria are inherited from the mother. Mitochondrial inheritance is characterized by maternal transmission and extreme variability of the phenotype within the same family. The difficulty of correlating phenotype with genotype in mitochondrial diseases means that genetic counseling and prenatal diagnosis for these conditions is highly problematical.

Sex Chromosome Abnormality

The common sex chromosome abnormalities are 47,XXY (Klinefelter's syndrome), 47,XXX (triple X), 45,XO (Turner's syndrome or monosomy X), and 47,XYY. Turner's syndrome, by far the least common in liveborns, is the one most likely to be encountered by the pathologist because over 99% abort spontaneously. It is the commonest chromosome abnormality among spontaneously aborted second trimester fetuses (Kalousek et al. 1990; Warburton et al. 1991). The typical phenotype is described in Chromosome Abnormalities on p. 154.

The other sex chromosome abnormalities listed above are phenotypically normal at birth and likely to be encountered by pathologists only if a termination of pregnancy is requested after an incidental finding of one of these abnormalities at chorionic villus sampling (CVS) or amniocentesis. Most liveborn XXY males present in adulthood for investigation of infertility; girls with Turner's syndrome may be recognized at birth because of typical phenotype, during investigation of short stature or heart murmur in childhood, or at puberty with primary amenorrhea. Most XYY males and XXX females remain undiagnosed.

Multifactorial Disorders

Multifactorial inheritance suggests that a disorder is caused by the interaction of several adverse genes and environmental factors. Under this heading fall disorders that occur with increased frequency among family members of an affected individual in an inverse frequency to their relationship. Families share environments as well as genes, and a thesis of environmental agents acting on a susceptible population was proposed (Carter 1963, 1969). A mathematical model invoking a threshold effect can be constructed, and recurrence risks in the offspring of family members calculated. Mapping of the multiple genes involved is difficult (Brombezzi et al. 2003). Common congenital malformations where this model of inheritance has been invoked include cleft lip and palate and neural tube defects, cardiac defects, and dislocation of the hip. The protective effect of folic acid administration to women who have borne a fetus with neural tube defect (Medical Research Council 1991a) lends support to this model.

Environmental Teratogens

From recognized environmental teratogens, such as rubella infection or particular drugs, we can extrapolate to a much larger number of agents with the potential to adversely affect the human embryo or fetus. Demonstration of a causal relationship in respect to a putative agent is very difficult because of the multiplicity of compounding

variables. These include the timing of the insult, the dose, interaction with other potential teratogens, and individual susceptibility. Initial recognition of a teratogen is only likely to occur when the effect is strong, or when it produces an unusual defect in sufficient cases. Recognition is most likely when there is a particular geographical or temporal restriction that highlights the problem. See the Eurocat special report, "The Environmental Causes of Congenital Anomalies: A Review of the Literature" (www.eurocat.ulster.ac.uk/pubdata).

Maternal Disorders

A variety of maternal diseases, either genetic or acquired, and deficiency states may directly affect the developing embryo. In other disorders, such as epilepsy, it is most likely that damage is attributable to therapy. Maternal phenylketonuria (PKU) is the best-documented example of genetic disease in the mother affecting her offspring when maternal serum phenylalanine level is elevated during pregnancy. The children of women with PKU who do not maintain a strict diet throughout pregnancy are at high risk for severe mental retardation with microcephaly and heart defects. It is likely that the elevated phenylalanine level acts during both the embryonic and fetal periods. The effect is mental retardation, unrelated to the genotype of the child (Levy and Ghavami 1996).

Maternal connective tissue disorders, such as osteogenesis imperfecta and Ehlers–Danlos syndrome, are risk factors for early amnion disruption syndrome leading to a variety of disruptions and deformity in the fetus (Young et al. 1985).

Maternal myasthenia gravis has been implicated as a rare cause of arthrogryposis multiplex congenita (Vincent et al. 1995).

Maternal type 1 diabetes (insulin-dependent diabetes mellitus; IDDM) is associated with an increased incidence of congenital abnormality in offspring of 6% to 9% (two to three times higher than for non-IDDM). The risk is related to the quality of blood glucose control at the time of conception. Defects of the heart, great vessels, kidneys, and neural tube are particularly common. Caudal regression, classically associated with maternal IDDM, is very rare. It is clear that improved control of the diabetic state using glycosylated hemoglobin levels as well as blood sugar monitoring has reduced the incidence of malformation in the offspring of these women (see Chapter 24).

Pregnant women with systemic lupus erythematosus (SLE) are at increased risk for miscarriage and (for those with anti-Ro antibodies), for the development of fetal heart block.

Drugs

Drugs with a known teratogenic effect are relatively few. Examples include alcohol, angiotensin-converting enzyme (ACE) inhibitors, carbimazole, cocaine, thalidomide, lithium, retinoic acid, warfarin, and anticonvulsant drugs (Koren et al, 1998).

Reliable prediction of drug teratogenicity in humans is not possible. It is important to remember that the effect of a drug (or lack of it) in animals is not direct evidence of its likely effect in humans.

Prescribed Medication

The action of some drugs such as antimetabolites and alkylating agents used as chemotherapeutic agents in the management of malignant disease make a teratogenic effect likely. This has been observed with a variety of antineoplastic drugs; folic acid antagonists such as aminopterin have the highest risk. Central nervous system defects and renal and limb abnormalities are well recognized, and there is an increased risk of early abortion with these drugs.

Women with epilepsy on treatment with anticonvulsant drugs have a two- to threefold increase in risk for a baby with a malformation. Those taking more than one antiepileptic drug are at higher risk. None of the established medications are without risk; sodium valproate is associated with the highest risk [central nervous system (CNS) and cardiac defects, spina bifida, and developmental delay] (Morrow et al. 2006); phenytoin is particularly associated with facial clefts and cardiac and genitourinary anomalies; carbamazepine (the safest of the established antiepileptic drugs) is associated with neural tube defects. The management of epilepsy in pregnancy is complex, as convulsions following withdrawal or reduction

of therapy may cause fetal death from hypoxia (see Holmes 2002).

Coumarin anticoagulants, for example warfarin, can affect both embryo and fetus. Embryopathic effects are chondrodysplasia punctata and nasal hypoplasia, while their effect on the fetus can result in microcephaly, mental retardation, and optic atrophy (Hall et al. 1980).

Vitamin A (retinol) affects neural tube development and closure in animals. There is increasing evidence that vitamin A and related compounds in high doses can produce malformations in humans. Craniofacial anomalies, hydrocephaly, posterior fossa cyst, cardiac and renal defects, and thymic hypoplasia are described. The facial appearance and related defects are similar to those described in DiGeorge syndrome. Retinoids are available without prescription and currently advertised for treatment of common skin disorders such as acne. Some compounds have a half-life measured in weeks or months, so there is a risk to pregnancies conceived after cessation of treatment (Lammer et al. 1985).

Diethylstilboestrol is both a prenatal carcinogen and teratogen, but there is little evidence to implicate other female sex hormone–like substances. Concern about the potential teratogenic effect of combined oral contraceptive pills in early pregnancy has abated.

Recreational Drugs, Alcohol, and Tobacco

Adverse effects on the offspring of women who abuse ethyl alcohol have been recognized for very many years. A variety of effects were described early last century (Ballantyne 1902), and during the last three decades the pattern of dysmorphic features and major malformations (fetal alcohol syndrome) has been recognized (Jones et al. 1973). These comprise microcephaly, hypoplasia of the midfacial skeleton with narrow palpebral fissures, hypertelorism, broad upturned nose, long philtrum, and smooth upper lip border. Microcephaly, cleft palate, and atrial or ventricular septal defects are common. Growth restriction, neonatal jitteriness, and failure to thrive are frequent problems. It seems likely that either periodic binge drinking or regular alcohol consumption at a level of about two units per day in early pregnancy can induce recognizable but mild abnormalities (Royal College of Obstetricians and Gynaecologists 2006).

Despite extensive study of the effects of smoking in pregnancy, there is no clear evidence to support a teratogenic role for tobacco when alcohol ingestion and other factors are controlled for. The adverse effects of smoking on fetal growth is quite clear, as is its relationship to spontaneous abortion, premature onset of labor, and stillbirth.

There is no evidence of a teratogenic effect of opiates or marijuana, but cocaine affects fetal vasculature and can result in disruptive defects, including limb reduction defects and intestinal atresia (Hoyme et al. 1990). The risk of anomaly to infants of cocaine-abusing mothers is probably not as great as previously thought (Hume et al. 1997).

Infection

A number of infectious agents [e.g., rubella, varicella-zoster virus, human immunodeficiency virus (HIV), parvovirus B19, *Toxoplasma gondii*, cytomegalovirus (CMV)] can affect the fetus, producing a range of effects from structural anomalies to immunological and hematological disorders and mental retardation. Rubella virus is embryopathic as well as having a recognizable fetopathic effect. Evidence for embryopathic effects of other infectious agents is not so clear-cut, and it is likely that some are lethal to the embryo, but the effect reduces as pregnancy progresses, being compatible with continued survival. The effects of intrauterine infection are discussed in detail in Chapters 4 and 16.

Physical Agents

Heat

Sources of elevated environmental temperature are maternal pyrexia for any reason, and external sources such as thermal baths or saunas. It is difficult to dissociate the effects of fever during maternal illness from a direct effect of the infectious agent on the embryo or fetus. In some laboratory animals, hyperthermia may interfere

with cranial neural tube closure. There is little evidence for this in humans, but it has been suggested that hyperthermia may cause Möbius syndrome (Graham et al. 1988), microcephaly, microphthalmia, as well as neuronal migration defects.

Radiation

Ionizing radiation has mutagenic and carcinogenic effects. Radiation at levels used for diagnostic purposes are associated with minimal risks. Therapeutic doses can cause microcephaly and increase the risk for childhood cancer and leukemia. Exposure before conception may increase the risk of both numerical and structural chromosomal abnormalities, as has been established in mice. The premeiotic divisions in the male are particularly sensitive.

Ultrasound

When ultrasonographic examination was first introduced into obstetric practice, much concern was expressed about possible disruptive effects on the embryo or fetus. Widespread and repeated use at all stages of gestation suggests that these early fears may be unfounded, but its absolute safety is unproven (Paneth, 1998).

Pregnancy Reduction

Because of the high incidence of preterm labor with consequential perinatal mortality and morbidity, fetal reduction using intracardiac potassium chloride may be offered in higher order multiple pregnancies, or selective termination when one fetus is discordant for an anomaly. In severe twin-to-twin transfusion syndrome, cord occlusion is the method. An anencephaly-like syndrome in surviving fetuses following embryo reduction is described (Boulot et al. 1990). The authors suggest a direct effect of the hypertonic saline (a method that is no longer used) used for reduction.

Amniotic Bands

Fetal disruption may result from direct pressure, entanglement, or ischemia because of the presence of amniotic bands (see Amnion Disruption Sequence p. 155).

Prenatal Diagnosis

It is now 35 years since the first prenatal diagnosis of Down syndrome was made following amniocentesis, and 30 years since the introduction of ultrasound scans to diagnose fetal anomalies. Recent advances in prenatal diagnosis include better screening programs and earlier and more accurate definition of fetal anatomy. A pregnancy may be known to be at high risk of abnormality because of a particular family history, leading to an appropriate search for a specific condition. Higher risk groups for chromosome anomaly include older mothers, those with a chromosomally abnormal child, or where one parent is a translocation carrier. These women may be offered CVS or amniocentesis. An increasing number of single-gene disorders are now identified at the molecular level, for example, Duchenne muscular dystrophy, cystic fibrosis, myotonic dystrophy, and fragile X, and for most families at risk for these disorders, prenatal diagnosis by analysis of DNA from CVS or amniocentesis sample is feasible. For families at high risk for chromosome abnormality or genetic disease, preimplantation genetic diagnosis (PGD) is becoming increasingly available. For a review of PGD, see Kuliev and Verlinsky (2005).

There is now a large number of population screening programs to identify women at increased risk of fetal abnormality. Screening for Down syndrome may be offered in the first trimester (e.g., nuchal scan combined test) or the second trimester (e.g., Triple blood test). Women identified as being at higher risk are offered a diagnostic test—CVS or amniocentesis. Women with elevated serum α-fetoprotein (AFP) levels need detailed ultrasound examination to search for neural tube defects or other structural anomaly (see Table 6.4). The increasing use of routine ultrasound scanning at 18 to 20 weeks' gestation (the anomaly scan) identifies many structural abnormalities in women previously thought of as being at low risk.

The different tests and screening procedures commonly in use are outlined below. For a more detailed review of prenatal diagnosis, see Milunsky (2004).

Ultrasound Examination

First Trimester

More anomalies are being detected earlier in gestation because of nuchal translucency (NT) scanning performed as part of a screening test for Down syndrome (see below). It involves measuring the area at the back of the fetal neck at 11 to 14 weeks' gestation (Fig. 6.1). An increased nuchal measurement indicates an increased risk for Down syndrome, as well as some other chromosome anomalies, structural anomalies (particularly cardiac defects), and some rare genetic syndromes (Souka et al. 2005). The mechanisms by which some abnormalities give rise to this transient anatomical change (NT) are poorly understood (Haak and van Vugt 2003).

Scanning in the first trimester also allows accurate dating of the pregnancy [best performed at 10 to 13 weeks' gestation; see National Institute for Clinical Excellence (NICE) guidelines (www.NICE.org.uk] and determination of chorionicity in multiple pregnancies. This is important because monochorionic twins are at higher risk for congenital anomalies than dichorionic twins and can develop twin-to-twin transfusion syndrome (TTS) (discussed in Chapter 12).

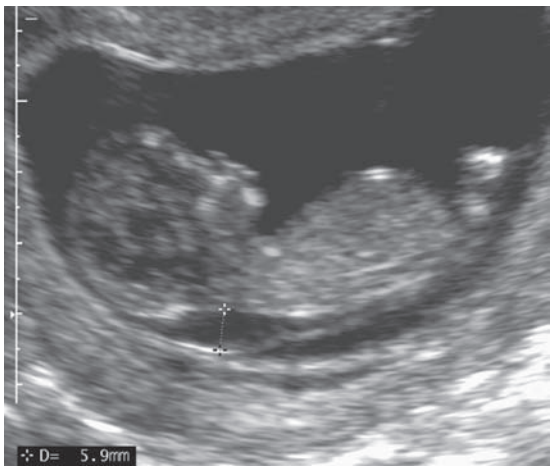


Figure 6.1. Nuchal translucency measuring 5.9 mm detected at booking at 12 weeks and 4 days. The conceptus is karyotypically normal, with no cardiovascular or other anomaly detected by 23 weeks' gestation. (Courtesy Dr. Jane Walker, Edinburgh.)

Second Trimester

The routine anomaly scan is performed at around 20 weeks' gestation. It is better at detecting some anomalies than others; for example, it detects most neural tube defects but is less accurate for cardiac anomalies. Some centers offer special scanning of the fetal heart at around 20 weeks for women at increased risk for a baby with a congenital cardiac abnormality because of, for example, a family history, increased nuchal translucency, or an abnormal four-chamber view on an anomaly scan. Fetal echocardiography will now detect most major cardiac defects. See Chitty and Yates (2004) for a review of fetal cardiac anomalies.

Some abnormalities are not usually suspected until later in gestation (e.g., hydrocephaly, microcephaly, tracheoesophageal fistula). Ultrasound surveillance is essential during the performance of invasive techniques (e.g., CVS, and for checking fetal viability before and after such procedures). Overall, around 60% of structural birth defects are detected prenatally, but the detection rate varies from 0% to close to 100% depending on the defect, for example, a 0% detection rate for isolated cleft palate and greater than 90% for gastroschisis. Wrong diagnoses are rare but false-positive diagnoses do occur; some are due to natural history (i.e., some regress during fetal life), but most are due to *ultrasound soft markers*, which are structural changes detected at ultrasound scan (mostly at the 20-week anomaly scan) that may be transient and in themselves have little or no pathological significance, but are thought to be more commonly found in fetuses with congenital abnormalities, particularly karyotypic abnormalities (Bricker et al. 2000). Examples are echogenic bowel, renal pelvic dilatation, and nuchal thickening. There is no general agreement on definition or management of these markers (such as when to report or when to offer invasive diagnostic test), and while reporting them has increased detection rates, this is at the expense of high false-positive rates. Table 6.1 shows the increase in prenatal detection rates in two 7-year periods and the effect of reporting ultrasound soft markers. Table 6.2 shows the detection rates for some common anomalies in an unselected population in Oxford (www.http:npeu.ox.ac.uk/carobb/).

Table 6.1. Changes in prenatal detection rates in two 7-year periods and the effect of reporting ultrasound soft markers

	1991–1997 (inclusive)	1998–2004 (inclusive)
Total births	40,494	38,661
% babies malformed at delivery	2.1 %	2.3%
% malformed babies detected prenatally	58%	75%
Number of cases with prenatal suspicion, baby normal at birth*	1 in 163	1 in 67

*Ultrasound soft markers.

Source: CAROBB (Congenital Anomaly Register for Oxfordshire, Buckinghamshire and Berkshire) report, 1991–2004 (Oxford data). http:npeu.ox.ac.uk/carrob/.

A recent evaluation of prenatal diagnosis in an unselected population found that 64% of congenital malformations obvious at birth were detected prenatally, mostly following initial suspicion during ultrasound scan (Boyd et al. 2005). In this study, 0.5% of all pregnancies resulted in termination because of prenatal diagnosis of fetal anomaly. The majority of pregnancies terminated had lethal anomalies or were associated with very severe morbidity (Table 6.3); 43% of fetuses were judged to have lethal anomalies, 14% possibly lethal, and 43% associated with possible survival but with long-term handicap.

Table 6.2. Prenatal detection of congenital abnormalities in the local unselected Oxford population, 1991–2004

Defect	Test performed	Number of pregnancies registered with prenatal suspicion of abnormality (not including false-positive diagnoses)	Number of cases registered with abnormality confirmed at delivery	Number of false-positive diagnoses	Prenatal detection rate
Isolated neural tube defects (anencephaly and spina bifida)	Ultrasound scanning +/– MSAFP	84	86[1]	1 (sacral teratoma)	98%
Isolated cardiac anomaly	Ultrasound scanning	52	163	0	31%[2]
Isolated cleft lip +/– palate	Ultrasound scanning	28	52	0	54%
Isolated clubfoot	Ultrasound scanning	57	113	9[3]	50%
Down syndrome	Karyotyping because MA at EDD >35 ($n = 29$) or nuchal/ biochemical screening ($n = 47$) or ultrasound scanning ($n = 48$)	124	198	0	63%
Isolated diaphragmatic hernia	Ultrasound scanning	12	22	4	63%[4]
Isolated exomphalos	Ultrasound scanning +/– MSAFP	11	16	2	69%
Isolated gastroschisis	Ultrasound scanning +/– MSAFP	16	16	2	100%

EDD, expected date of delivery; MA, maternal age; MSAFP, maternal serum α-fetoprotein screening.

[1]One declined screening.

[2]There is underreporting of cardiac anomalies diagnosed after discharge from the maternity unit therefore true prenatal detection rate is lower.

[3]Positional talipes at birth.

[4]Excludes three not scanned.

Source: CAROBB (Congenital Anomaly Register for Oxfordshire, Buckinghamshire and Berkshire) report, 1991–2004 (Oxford data). http:npeu.ox.ac.uk/carrob/.

Table 6.3. Termination of pregnancy for fetal anomaly in an unselected population: number of pregnancies terminated broadly categorized by defect and lethality

Main defect/system	Lethal		Possibly lethal		Possible survivor		
Defect	Abnormality	*n*	Abnormality	*n*	Abnormality	*n*	Totals
Neural tube defect	Anencephaly	26	Hydrocephalus/other	3	Spina bifida	30	66 (21%)
	Encephalocele	1					
Other CNS	Other	1			Other	5	
Chromosomes	Abnormal karyotype	53	Abnormal karyotype	8	Abnormal karyotype	80	141 (46%)
Cardiac	Hypoplastic left heart	4	Complex cardiac	3	Fallot	1	8 (3%)
Renal	Renal	8	Obstructive renal	2			10 (3%)
Skeleton (includes some inherited disorders)	Skeletal dysplasia	9	Skeletal	5	Skeletal limb reduction	2	16 (5%)
Anterior abdominal wall/diaphragm			Diaphragmatic hernia	4	Exomphalos	1	5 (2%)
Hydrops/cystic hygroma	Hydrops	3	Cystic hygroma	1	Cystic hygroma	2	6 (2%)
Multiple abnormalities		13		2			15 (5%)
Inherited disorders	Meckel Gruber syndrome	5	Myotonic dystrophy	5	Sickle cell	1	26 (8%)
	Multiple pterygium syndrome	1	Donohue syndrome	1	Duchenne MD	1	
	Orofaciodigital syndrome	1	SMA	4	Adrenogen syndrome	1	
			OTC	1			
			Unknown recessive	1	Anti-1-antitrypsin defic.	1	
					Unknown recessive	2	
					Fragile X	1	
Other	Amniotic bands, conjoined twins syndrome	8	Teratoma	2	Normally formed fetus*	2	16 (5%)
			? syndromes	2			
					Cystadenomatous lesion of lung	1	
					Cornelia de Lange syndrome	1	
Total		133 (43%)		44 (14%)		132 (43%)	309 (100%)

*see text.

MD, muscular dystrophy; OTC, ornithine transcarbolamine deficiency; SMA, spinal muscular atrophy.

Source: CAROBB (Congenital Anomaly Register for Oxfordshire, Buckinghamshire and Berkshire) report, 1991–2004 (Oxford data). http:npeu.ox.ac.uk/carrob/.

Invasive Tests

Chorionic Villus Sampling

Although first performed in the late 1960s CVS, became widely available only in the 1990s, when better ultrasound equipment and improved monitoring of the procedure enabled the development of techniques with a lower risk of complication.

The overwhelming advantage of CVS over amniocentesis is that it can be carried out during the first trimester with, in the event of a positive diagnosis, earlier termination of pregnancy. For many women this has made possible the decision to attempt another pregnancy.

Most CVSs are carried out transabdominally, the transcervical route being used if the placenta is inaccessible transabdominally. Because of concern about the safety of CVS carried out early in gestation, most centers now carry out this procedure after 11 weeks of gestation; 5 to 25 mg of

chorionic villi are obtained by aspiration or biopsy under ultrasound guidance. Karyotyping can be performed from both direct examination or short-term culture of the cytotrophoblast and long-term culture of fibroblasts from the villus core. Rapid karyotyping of common trisomies by FISH or polymerase chain reaction (PCR) is now commonly used.

Indications for Chorionic Villus Sampling

1. Diagnosis using DNA techniques, for example, cystic fibrosis, Duchenne muscular dystrophy, and fragile X. Chorionic villi are a good source of fetal DNA and are generally preferred to amniocytes because of the greater volume of tissue that can be obtained.
2. Inborn errors of metabolism, when the enzyme in question is known to be expressed in chorionic villi (see Chapter 7).
3. Karyotyping, for example, advanced maternal age for trisomies as a primary indication is now being replaced by higher risk first trimester screening result (see Ultrasound Examination: First Trimester p. 132), familial translocations, following an abnormal scan.

Chorionic villus sampling is the method of choice for DNA diagnoses.

Complications of Chorionic Villus Sampling

- Maternal cell contamination <1% if villi are selected by microscopy.
- Confined placental mosaicism (Gardner and Sutherland 2004), where the chorionic villi have a different karyotype from the fetus in 1% to 2% of specimens at 10 to 11 weeks' gestation. It is more common in direct (cytotrophoblast) preparations. It can lead to false-positive diagnoses (CVS abnormal, fetus normal) and false-negative diagnoses (CVS normal, fetus abnormal). The risk of wrong diagnosis is reduced considerably if both direct (cytotrophoblast) and long-term cultures (extraembryonic mesoderm) are karyotyped. The finding of a usually nonviable karyotype at CVS is an indication for amniocentesis. In most cases this will reveal a normal fetal karyotype.
- Miscarriage risk: Two randomized clinical trials, the Canadian Multicentre Trial and the Medical Research Council (MRC) European Trial, compared the fetal loss following CVS to amniocentesis (Canadian Collaborative CVS-Amniocentesis Clinical Trial Group 1989; Medical Research Council 1991b). Both reported increased fetal loss in the CVS group, with the MRC European trial reporting the higher loss rate. The European results show that a woman allocated CVS had a 4.6% (confidence limits 1.4% to 7.8%) less chance of a successful pregnancy outcome than a woman allocated to second trimester amniocentesis (see Royal College of Obstetricians and Gynaecologists 2005 for a discussion of risks associated with CVS and amniocentesis).
- Fetal injury: Initial studies concentrated on miscarriage risk rather than abnormalities of babies born following CVS, and there are no long-term follow-up studies. However, there followed isolated reports of abnormalities associated with CVS (Boyd et al. 1990; Fig. 6.2) and subsequently of four babies with oromandibular/limb hypogenesis syndromes following a CVS performed at less than 10 weeks' gestation (Firth et al. 1991). This report provoked reviews of the

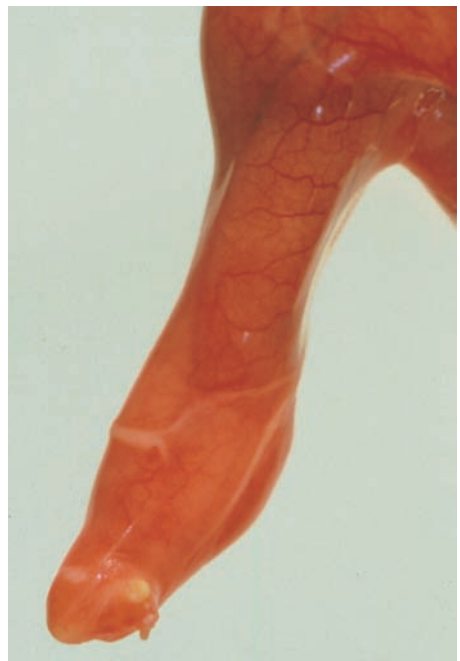


Figure 6.2. Transverse limb defect seen on scan at 17 weeks' gestation. Chorionic villus sampling (CVS) performed at 9 weeks (Keeling 1994).

experience in other centers, several of which confirmed an increase in the number of cases of oromandibular/limb hypogenesis syndromes and transverse limb reduction defects (reviewed by Firth 1997). Quintero et al. (1992) visualized hemorrhagic lesions that appeared on the calvarium, thorax, face, and limbs by embryoscopy during CVS after 9 weeks' gestation. A systematic review has addressed this and other factors associated with congenital limb defects (Brown et al. 1996), and concluded that there is good evidence for an association between CVS and the occurrence of congenital limb reduction defects identified in both cohort and case—control studies. The earlier the procedure is carried out, the stronger the association and the greater the likelihood of a more severe defect. The need for follow-up after prenatal procedures and for careful documentation is emphasized by these reports.

Amniocentesis

Amniocentesis has been performed for much longer than CVS and is a more routine procedure; 10 to 20 mL of amniotic fluid are taken under ultrasound guidance transabdominally usually between 15 and 18 weeks' gestation.

Late second- and third-trimester amniocentesis may be performed for karyotyping following a late scan diagnosis of abnormality (e.g., suspicion of duodenal atresia due to a "double bubble" appearance) or to assist for amniotic fluid reduction in the case of severe polyhydramnios. The safety and adequacy of early amniocentesis, from about 10 weeks' gestation, has been assessed and found to be associated with a higher risk for miscarriage than CVS and increased risk for clubfoot [Canadian Early and Mid-Trimester Amniocentesis Trial Group (CEMAT) 1998; Royal College of Obstetricians and Gynaecologists 2005].

Indications for Amniocentesis

The indications are similar to those for CVS. In addition, sampling may be performed for AFP and acetylcholinesterase isoenzymes for diagnosis of neural tube defects (NTD) in cases with positive family history or raised serum AFP, although nearly all centers now rely on ultrasound scanning to diagnose NTD. Amniocentesis may also be performed following diagnosis of abnormality by ultrasound scan to enable karyotyping to be undertaken. The majority of amniocenteses are performed because of increased risk of Down syndrome following a screening test. The United Kingdom National Screening Committee now recommends that all women are offered screening for Down syndrome and that amniocentesis solely on the basis of maternal age be discouraged.

Complications of Amniocentesis

- Reliability: amniotic fluid cell culture is a very accurate means of providing a fetal karyotype (Gardner and Sutherland 2004). Maternal cell contamination occurs in 0.5% to 1% of cultures, and in vitro chromosome changes may give rise to pseudomosaicism.
- Miscarriage risk after amniocentesis has been well studied [National Institute of Child Health and Human Development (NICHD) National Registry for Amniocentesis Study Group 1976; Canadian Medical Research Council 1977; Medical Research Council 1978; Tabor et al. 1986]. A miscarriage rate of 0.5% to 1.4% was found in these large studies.
- Fetal damage during amniocentesis is very rare, particularly in experienced hands under ultrasound control, but has been documented. Limb abnormalities may be the result of direct fetal damage (Lamb 1975) or secondary to membrane damage (Rehder 1978) and amniotic bands in the case of distal constrictions and amputations. Injury to thoracic organs (Rushton 1981), abdominal viscera (Swift et al. 1979; Therkensen and Rehder 1981) (Fig. 6.3), and brain (Squier et al. 2000; Villo et al. 2001) has been described. When intrauterine fetal death follows such injury, the puncture site may be difficult to identify because of supervening maceration, and the possibility of injury is raised only by the nature of the internal abnormality.

Fetal Blood Sampling

The use of the fetoscope to obtain fetal blood samples from the umbilical cord under direct vision has been superseded by ultrasound-guided transabdominal fetal blood sampling (FBS) after 18 weeks' gestation. Fetal blood sampling is carried out less frequently now that rapid karyo-

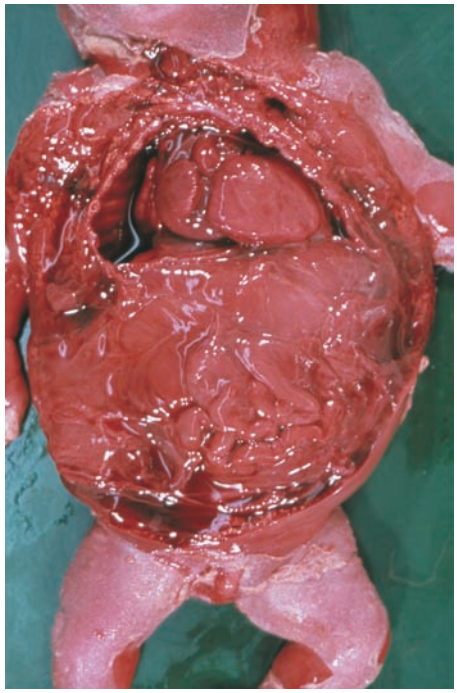


Figure 6.3. Abdominal distention and peritonitis with adhesions; intrauterine death after amniocentesis.

typing by FISH or PCR is available from CVS and amniocentesis samples, but it is still required for the treatment of rhesus disease and fetal anemia following diagnosis by middle cerebral artery Doppler studies.

Other Invasive Tests/Fetal Surgery

Successful treatment has been done for spina bifida, diaphragmatic hernia (fetal tracheal occlusion; see Deprest et al. 2004), cystic adenomatoid malformations, obstructive uropathy, and cardiac anomalies. However, many of the treatments are controversial, and randomized controlled trials are needed to evaluate them. Aspiration and analysis of urine in male fetuses in some cases of urethral obstruction, usually due to urethral valves, together with careful renal ultrasound, can provide useful information regarding long-term prognosis and possible shunting procedures. The outlook is usually poor and the benefit of shunting questionable. Laser ablation of communicating vessels in pregnancies complicated by twin-to-twin transfusion is now performed more widely.

Prenatal Diagnosis and DNA Analysis

Prenatal diagnosis of many rare single-gene disorders has been revolutionized by the accessibility of DNA analysis. The genes for many of the most common single-gene defects have now been identified. The gene in question may have been cloned and sequenced, and some mutations causing the disorder characterized. The hemoglobinopathies, cystic fibrosis, myotonic dystrophy, spinal muscular atrophy, achondroplasia, fragile X, Huntington's chorea, and Duchenne muscular dystrophy are among the more common disorders in which this has been achieved. Many other disorders for which the gene has not yet been isolated have been mapped to a particular region of a chromosome. Prenatal diagnosis using linked DNA markers is possible for families at risk of these disorders when DNA is available from appropriate family members. This includes fetuses from previously terminated pregnancies. The importance of storing tissue from any fetus at risk of single-gene disorders cannot be overemphasized.

Prenatal Diagnosis and Cystic Fibrosis

Since identification of the cystic fibrosis (CF) gene in 1989, CF is known to be due to mutations in the CF transmembrane conductance regulator gene (*CFTR*) located on chromosome 7q31. More than 1000 mutations have been identified in *CFTR*, the most frequent of which, a 3-base-pair (bp) deletion, leads to the absence of a phenylalanine residue at position 508 (F 508). This mutation occurs in approximately 70% of north European carriers. The remaining 30% of carriers have a variety of mutations. Approximately 10% of carriers have mutations still to be characterized at the molecular level so that population screening for carriers is not straightforward (Rosenstein and Zeitlin 1998).

Accurate prenatal diagnosis is now possible for many families at high risk by direct mutation detection in DNA from CVS samples. If parents are known to be carriers but have a mutation not yet identified, prenatal diagnosis is usually possible, when DNA from the affected child is available, by gene tracking with intragenic and extragenic restriction fragment length polymorphisms (RFLPs).

The finding of echogenic bowel on prenatal scan gives a risk for cystic fibrosis in the fetus of 2% to 3%; parents may be offered testing to see if they are carriers. If they are both identified as carriers, prenatal diagnosis is offered. A negative result on screening for carriers reduces but does not entirely remove the risk for CF because 10% of carriers have mutations yet to be characterized (see above).

Prenatal Maternal Serum Screening for Neural Tube Defects

The observation by Brock and Sutcliffe (1972) of high levels of AFP in amniotic fluid of pregnancies complicated by open NTDs, followed by the finding that in most of these cases the maternal serum AFP (MSAFP) was also elevated, became the basis for an effective screening test for NTDs in high-incidence areas (Ferguson-Smith 1983). With increasing accuracy of ultrasound diagnosis, maternal serum screening of AFP solely for identification of NTDs has been superseded. However, because AFP is a component of the triple blood test (second trimester screening test for Down syndrome), should a high AFP level be identified, a detailed ultrasound scan is recommended to search for a neural tube defect or other anomaly associated with a raised maternal serum AFP (Table 6.4).

Antenatal Screening for Down Syndrome

Originally, screening for Down syndrome (DS) was based on the knowledge that the incidence of DS increases with maternal age (Hook 1990). Theoretically, if all women over the age of 36 years had amniocentesis performed for karyotyping, approximately 30% of DS pregnancies would be detected. An increasing number of screening tests now involve ultrasound (NT scan) and maternal serum biochemistry to identify those women at higher risk of having a baby with Down syndrome. The U.K. National Screening Committee (NSC) has addressed the inequity in the provision of screening tests and made a series of recommendations. All centers in England and Wales must offer all women screening for DS. Strict standards apply with initially a minimum detection rate of 60% for a 5% false-positive rate (FPR). A set timetable for increase in detection rate, reduction in FPR, and the offer of first-trimester screening (NT plus biochemistry) will follow. The latest information on detection rates and FPR by different screening methods is given in the Serum, Urine, and Ultrasound Screening Study (SURUSS) report from which Table 6.5, showing the FPRs for an 85% detection rate of the various tests available, is adapted. Malone et al. (2005) compares first and second trimester or a combination of both methods of screening for DS.

Future Developments in Prenatal Diagnosis

See Kumar and O'Brien (2004) for a recent discussion of future developments in prenatal diagnosis.

Earlier Diagnosis

There is always pressure to diagnose fetal abnormality at the earliest opportunity. Although there is an obvious advantage to the pregnant woman in knowing as early as possible if an abnormality is present, caution is needed because earlier tests may be less safe or less accurate (see Chorionic Villus Sampling, above, and Amniocentesis).

First-trimester ultrasound has yet to be fully evaluated. Some of the pregnancies identified would have miscarried naturally; perhaps 40% of chromosomally abnormal fetuses miscarry after 11 weeks' gestation. There is probably less psychological morbidity following miscarriage than opting for termination, and it may be wasteful of resources to diagnose abnormalities and carry out termination on pregnancies destined to miscarry. Less comprehensive information from an autopsy following first-trimester termination of pregnancy may mean that accurate information on recurrence risks is not be possible (Chitty and Pandya 1997).

Research into new markers to further refine screening tests for chromosomal abnormalities is ongoing. Presence/absence of the fetal nasal bone, of tricuspid regurgitation and normal/abnormal Doppler velocity waveform in the ductus venosus all have potential as first trimester ultrasound markers.

Novel Imaging Techniques

Fetal imaging techniques continue to evolve, and as they become more affordable and easier to

Table 6.4. Situations associated with raised levels of α-fetoprotein in maternal serum or amniotic fluid in the second trimester of pregnancy

Elevated α-fetoprotein	Pathogenesis	Association	Reference
Maternal serum	Physiological:		
	increased production	Multiple pregnancy	Wald et al. (1978)
	Pathological:		Stirrat et al. (1981)
	loss of placental surface integrity	Abdominal pregnancy	
		Fetomaternal hemorrhage	
		Placental angioma	Mann et al. (1983)
Maternal serum, amniotic fluid	Interruption of cutaneous integrity	Neural tube defects	Brock and Sutcliffe (1972)
		Omphalocele	Laurence (1982)
		Gastroschisis	Laurence (1982)
		Amnion rupture sequence	Aitken et al. (1984)
		Meckel syndrome	Chemke et al. (1977)
		Scalp defect (trisomy 13)	Fitzsimons et al. (1976)
	Altered skin permeability	Missed abortion	Wisniewski et al. (1974)
	Altered skin permeability	Teratoma	
	+ ? local pressure effect	Cystic hygroma (45XO)	Seller et al. (1974)
		Fetal hydrops	
		Urethral (valvular) obstruction	Vinson et al. (1977)
		Urethral atresia	Nevin et al. (1978)
		Hemangioma of umbilical cord	Barson et al. (1980)
	Increased urine production	Monoamniotic (conjoined) twins	Seller et al. (1977)
	Defective renal function	Congenital nephrotic syndrome	Kjessler et al. (1975)
		Polycystic kidney	Koontz et al. (1983)
	Defective fetal swallowing and α-fetoprotein breakdown	Esophageal atresia	Seppala (1973)
		Duodenal atresia	Weinberg et al. (1975)
		Hydrocephalus	
		Anencephaly	
	Concentration because of oligohydramnios	Renal agenesis	Balfour and Laurence (1980)
	Large/abnormal placental surface	Triploidy	

apply, the most useful will become incorporated into general clinical use. Three-dimensional ultrasound has potential for further evaluation of facial dysmorphism and complex congenital heart defects and prenatal fetal magnetic resonance imaging (MRI) for elucidation of intracerebral pathology beyond that possible with ultrasound. Ogle and Rodeck (1998) review these and other imaging techniques.

Laboratory Advances

Traditionally, samples have been cultured and the full karyotype analyzed, which can take 2 to 3 weeks. Newer techniques include interphase FISH, where appropriately labeled DNA probes are used to diagnose the common trisomies such as 13, 18 and 21, or microdeletions such as that on the long arm of chromosome 22 (DiGeorge/velocardiofacial syndrome), and quantitative fluorescent polymerase chain reaction (QFPCR). These tests can give results within a day or two. Accuracy, particularly regarding confined placental mosaicism is still being assessed. There is currently a debate about whether these rapid techniques, giving information only on specific abnormalities such as DS, will replace full culture karyotype in DS screening programs (Chitty et al (2006).

Table 6.5. The main antenatal screening tests for Down syndrome, usual gestation at test, and false-positive rate for 85% detection rate

Screening test	Gestation (weeks) of pregnancy when tests performed	False-positive rate (%) (95% confidence interval) for 85% detection rate
First trimester		
NT scan	12–13	20 (18.6–21.4)
Combined test (NT scan plus free β-hCG & PAPP-A)	10–13	6 (5.8–6.6)
Second trimester		
Triple blood test (AFP, uE3, free β-hCG)	15–18	9.3 (8.8–9.8)
Quadruple test Same as triple plus inhibin-A	15–18	6.2 (5.8–6.6)
First and second trimester		
Integrated test (NT scan plus PAPP-A and quadruple test) (only one result given—after integration of first and second parts of test)	11–13 and 15 weeks	1.3 (1.2–1.4)

hCG, human chorionic gonadotrophin; NT, nuchal translucency; PAPP, pregnancy-associated plasma protein; uE3, estriol.
Adapted from Wald et al. (2003).

The ultimate aim of a test is to have 100% accuracy and no risk to the mother or baby. The search for fetal cells in maternal blood is ongoing (Bischoff et al. 2003). It is possible to determine fetal blood groups from maternal blood samples through detection of free fetal DNA, which is starting to be used in the management of rhesus disease and for noninvasive sexing of fetuses at risk for X-linked conditions so that invasive diagnostic testing can be offered if the at-risk fetus is male.

Examination of the Fetus and Placenta

When pregnancy terminates prematurely, either electively or spontaneously, following investigations for or diagnosis of fetal malformation, pathological examination of the fetus is essential. There are three important reasons for undertaking fetal examination. The first is confirmation of the nature of the abnormality for which termination of pregnancy was undertaken. The second is careful scrutiny of the fetus, placenta, and membranes for any abnormality that might be related to invasive investigations. Both of these points merit attention; together they comprise the quality control of antenatal diagnosis of malformation and are an essential, but often neglected, part of the antenatal diagnostic facility. The third and perhaps most important reason for fetal examination is the careful documentation of all abnormalities present. It is the combination of external dysmorphic features and other internal abnormalities accompanying a major structural anomaly identified prenatally that allows precise recurrence risks to be calculated. In some cases the contribution made by careful documentation of the whole picture may be the difference between no increase in risk of recurrence and a risk of one in four in every subsequent pregnancy.

Value of Fetal Examination

There is great variation in the accuracy of methods of prenatal diagnosis. Molecular, cytogenetic, and

biochemical techniques usually allow very precise diagnosis, although sampling may introduce errors. Some methods of diagnosis are less specific. Ultrasound examination will demonstrate a major defect but may not identify it precisely (Fig. 6.4), nor will it identify many of the minor dysmorphisms that are necessary for syndrome diagnosis. Rutledge et al. (1986) compared ultrasound diagnosis with pathological findings and found an average of two additional malformations per case. They were unable to confirm some prenatal diagnoses. It is clearly most important to examine the fetus when conditions for ultrasound examination are suboptimal, for example when there is oligohydramnios or in the third trimester when fetal position is relatively fixed. Manchester et al. (1988) followed 257 pregnancies referred for detailed ultrasound because of prenatal suspicion of anomaly. Forty-six pregnancies were electively terminated, and postnatal evaluation, clinical or postmortem or both, was undertaken in all cases. False-positive diagnoses were made in 1.5% and false-negative diagnoses in 2% of cases. Additional anomalies were present in 37% of malformed infants. Shen-Schwartz et al. (1989) found that postmortem examination of the fetus yielded clinically useful information in 46% of their cases. The pathologist's contribution was more likely where multiple malformations were present.

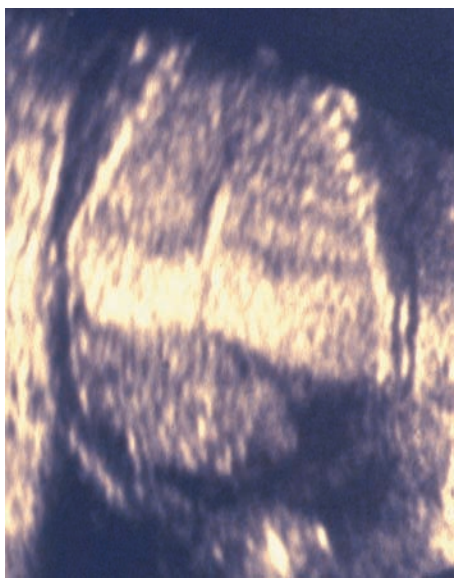


Figure 6.4. Ultrasound scan of fetal trunk. There is edema and ascites, diaphragms depressed, bright lungs. (Courtesy of Dr. B.B. Muir, Edinburgh.)

In a retrospective necropsy study Chescheir and Reibauer (1994) found that prenatal ultrasound examination permitted identification of 61% of all congenital malformations found at autopsy and 87% of major anomalies. False-positive diagnoses were minor (renal pyelectasis) or occurred in the presence of oligohydramnios or scanning after fetal demise. The majority of anomalies not identified were facial, cardiac, extremity, and genital anomalies. Another retrospective study (Goncalves et al. 1994) found that ultrasound examination at around 16 weeks' gestation detected 53% of defects and 89% of lethal malformations. Cardiac defects, microcephaly, and musculoskeletal anomalies were often not detected by scanning at that gestation.

Sun et al. (1999) examined the usefulness of fetal examination after termination for malformation in both intact fetuses and fragmented specimens. Examination of intact fetuses confirmed ultrasound findings in 65.5% of CNS disorders and in 47.5% when anomalies were present in other systems. Most new information was in fetuses with cardiovascular disorders. Not surprisingly, the disrupted fetuses were much less informative, and no prenatal diagnoses were challenged in that group.

Studies in the U.K. are similar. Clayton-Smith et al. (1990) found that fetal examination changed or refined ultrasound diagnoses in 40% of cases, and Keeling et al. (1990) found that 42% of prenatal diagnoses were modified or changed (Fig. 6.5) including 2% false-negative diagnoses. Even following diagnosis based on amniotic fluid sampling, clinically useful information may be derived from fetal examination. Clayton-Smith et al. (1990) found that it changed parental counseling in 3% of cases. Weston et al. (1993) found that necropsy examination in a specialist center revealed additional or different anomalies from those identified on ultrasound examination in 44% of cases. In 25% of their cases, this additional information altered patient management. In an evaluation of regionally based antenatal ultrasound examination, the main ultrasound

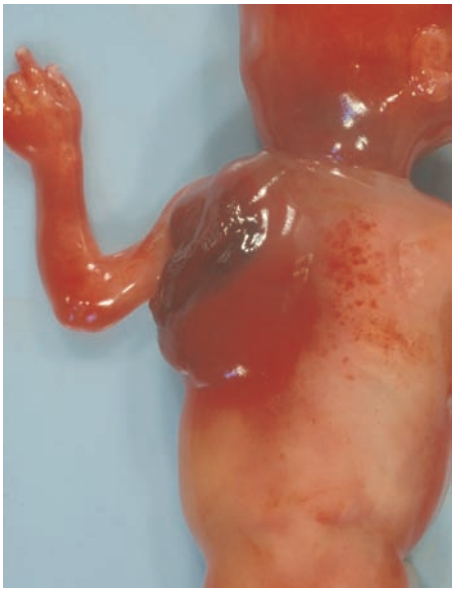


Figure 6.5. Eighteen-week-gestation fetus with a large dorsal angioma. The ultrasound diagnosis was meningocele.

findings were confirmed in 91% of cases and changed to an equally or more serious anomaly in 8.4% (Brand et al 1994). However, subsequent pathological examination resulted in change or refining of the diagnosis in 25% of cases.

Despite the improved definition of ultrasound images and the accumulated experience of radiologists and obstetricians, comparisons between ultrasound diagnoses and fetal examination continue to demonstrate that additional significant information is derived solely from fetal examination. Yeo et al. (2002) found complete agreement in 65% of their cases, but that only 18% of minor anomalies were detected. In a smaller study, Johns et al. (2004) found major agreement in half of cases examined. Significant additional findings were revealed by fetal examination in 28% of cases. Sankar and Phadke (2006) discovered additional abnormalities in 56% of their cases. These led to a change in recurrence risk for families in 17%.

Information for the Pathologist

To accomplish the aims of fetal examination from a pregnancy terminated following the diagnosis of congenital abnormality or inherited disease, the pathologist must have sufficient clinical information before the examination is begun to ensure that the right investigations are undertaken. The minimum information required is identification, maternal age, and past obstetric history, together with details of the terminated pregnancy including estimates of gestation; reasons for the investigation; the nature, dates, and results of scan reports; investigations undertaken with their results; the clinical diagnosis; and method of termination. This information should accompany the unfixed fetus and placenta, all of which should be transferred to the laboratory as quickly as possible after delivery. Optimally, the pathologist should be forewarned of all terminations of pregnancy undertaken for fetal anomaly, but this may be difficult in practice. However, this information is essential when confirmation of diagnosis demands recourse to techniques requiring fresh tissue or blood such as tissue culture, biochemical, or DNA analysis, particularly if liaison with other laboratories is necessary.

Fetal Examination

At present, the majority of terminations of pregnancy following the prenatal diagnosis of fetal anomaly take place between 17 and 20 weeks' gestation, and most of this section describes the examination that is appropriate for the fetus at this stage of development. First-trimester CVS is now the method of choice for the detection of many inherited disorders, with suction evacuation used to terminate the affected pregnancy. The embryo can be retrieved in good condition by suction with ultrasound guidance up to 10 weeks' gestation (Soothill and Rodeck 1994). Examination with a hand lens or dissecting microscope permits external examination and limited dissection. If the embryo is disrupted, tissues, limbs, and whole organs can be distinguished (Kalousek et al. 1990). It is usually possible to retrieve tissue to enable confirmation of the antenatal diagnosis to be undertaken, and in some cases histological and electron microscopic examination may be possible.

The fetus should be transported to the pathology department in a fresh state in a clean, dry container as quickly as possible following delivery. Immersion of the fetus in fixative rules out

the possibility of tissue culture and some biochemical studies, preventing confirmation of the working diagnosis. The presence of additional, prenatally undetected, defects increases the likelihood of a chromosome anomaly considerably (Kennedy et al. 1998).

Before dissection of the fetus is begun, weight and measurements should be recorded, anteroposterior (Fig. 6.6) and lateral radiographs obtained, and a photographic record of all external abnormalities made (Fig. 6.7). When termination has been undertaken for skeletal dysplasia, high-quality radiographs are essential and may be diagnostic. Examination of the axial skeleton may reveal distinctive abnormalities in the autosomal trisomies (Kjaer et al. 1996, 1997a; Keeling et al. 1997) and triploid fetuses (Kjaer et al. 1997b) (Fig. 6.8). It is sometimes necessary to sample tissue for culture before the fetus is photographed. This should be done as inconspicuously as possible; the axilla and inner thigh are the least intrusive sites.

Detailed external examination is important, with careful scrutiny and recording of each part of the body in an orderly fashion. The shape of the head, and the size and shape of the fontanelle, eyes, nose, mouth, palate, and ears and any neck webbing are recorded. The length and proportions of limbs, the form of hands and feet, together with the number, length, and curvature of digits, are recorded. The trunk is examined with particular attention given to the presence of abdominal wall defects. The back must be carefully examined, recording the size and site of any abnormality such as meningomyelocele or skin pitting. Careful observation of external abnormalities may suggest the possibility of a particular syndrome and change the significance of major malformations in respect to subsequent genetic counseling.

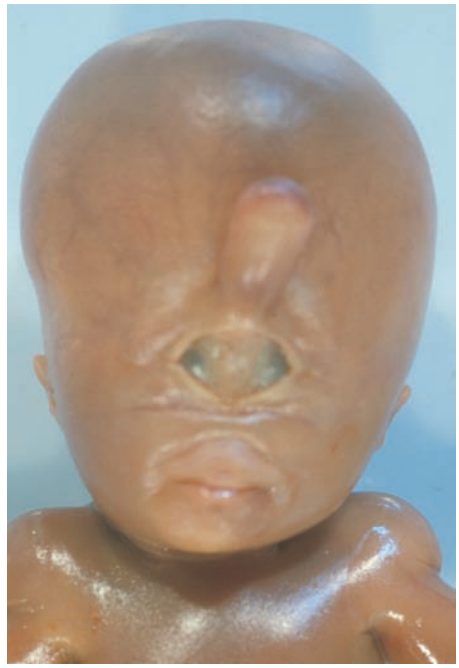


Figure 6.7. Facial dysmorphisms comprise supraorbital proboscis, synophthalmia, long philtrum, and smooth upper lip. The cranial vault is large, and holoprosencephaly was present.

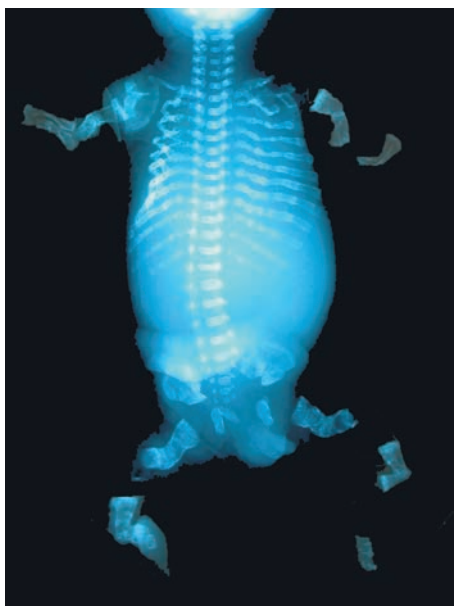


Figure 6.6. Radiograph of a 20-week-gestation fetus with osteogenesis imperfecta type II. There are multiple healing rib fractures, and fracture, deformity, and shortening of limb bones.

Artifactual fetal distortion or injury may result from vaginal delivery or subsequent handling. Membranous sacs such as meningomyelocele or omphalocele are particularly vulnerable to delivery trauma. Care should be taken to distinguish spurious "abnormalities" from developmental defects and in utero deformations (see Artifactual Abnormalities p. 145).

Internal examination of the midtrimester abortus follows a similar method to perinatal necropsy (see Chapter 2), with a particular search for

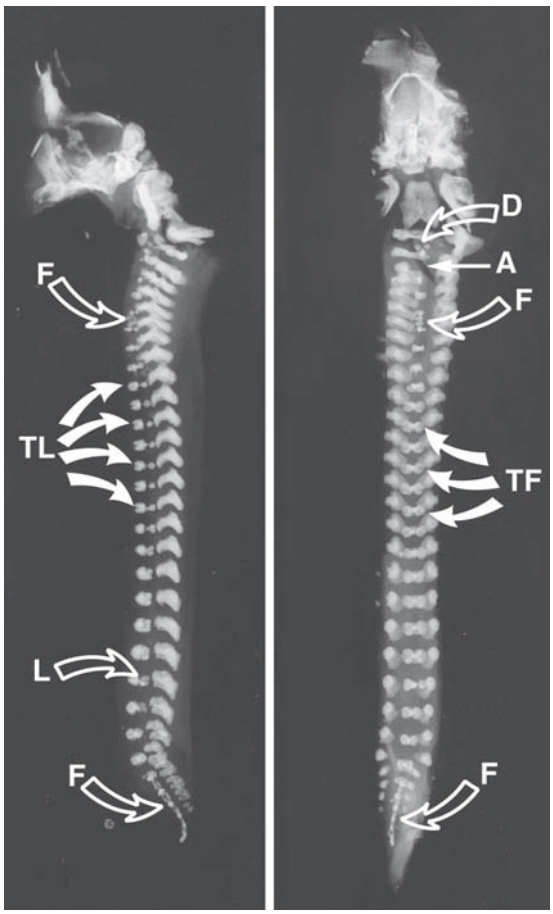


Figure 6.8. Radiograph of the vertebral column, 18-week fetus with trisomy 21. A, absence of cervical vertebral bodies; D, bifid vertebral body; F, fusion of adjacent vertebrae; TF, butterfly-shaped vertebral bodies; L malformed lumber vertebrae; TL, lateral view. (Keeling et al. 1997, with permission the *American Journal of Medical Genetics*.)

abnormalities expected from the clinical diagnosis. A detailed record is made of all abnormalities found. Histological examination of all major organs, particularly any that appear to be developmentally abnormal, should be undertaken.

Tissue samples such as skin, lung, and gonad should be transported in tissue culture medium for chromosome studies. Touch preparations of the cut lung surface onto glass slides can be evaluated using FISH techniques to confirm prenatally diagnosed karyotypic abnormality or for diagnostic backup when tissue preservation is poor. Samples for confirmation of genetic metabolic disease (see Chapter 7) need to be tailored to specific diagnoses, but skin and lung for fibroblast cultures are usually required, and organ samples for biochemical analysis should be snap frozen. A frozen sample of liver or spleen is useful for DNA studies, although an increasing number of investigations can now be performed on fixed tissue. It may be worth archiving an extra tissue block for this purpose. When termination of pregnancy has been undertaken for maternal infection such as rubella or varicella, fetal blood for viral antibody levels and organ and placental samples for virus culture should be attempted.

When invasive investigations have been undertaken, injury to the fetus, cord, and placenta should be carefully sought. The placenta, cord, and membranes should be examined. The appearance and amount of membrane recorded, discoloration from hemorrhage or infection, perhaps related to amniocentesis, and nodularity of the amnion as an indication of long-standing oligohydramnios, should be sought. Hematomas attached to the chorion or placenta or lying within placental tissue might be related to sampling procedures. Their appearance should be recorded and samples taken for histological examination to determine the age of the lesion.

Reconstruction and Disposal of the Fetus

Attitudes about the needs of the parents for support and an opportunity to mourn their aborted fetus have changed considerably (Elder and Laurence 1991). It is important to be aware before the examination begins of the parents' wishes concerning fetal disposal. The need to reconstruct the body in a sensitive fashion for return to the parents for burial or cremation may conflict with dissection methods used to best demonstrate some anomalies. It is also sensible to check that photographs of the fetus have been taken for the parents (Batcup et al. 1988). Some parents may take several weeks to decide whether or not they wish to arrange a funeral ceremony. Medical or nursing staff should keep the pathology department fully informed about any indecision of this nature.

Difficulties in reconstruction are frequent in small fetuses. The skin of the second trimester abortus is poorly keratinized, and collagen is immature so that sutures tend to cut out. Fine

linen thread or silk with a fine needle may suffice. Some authors report better cosmetic results using epoxy resin (Gau et al. 1991). This must be used with care, as the hardening reaction is exothermic and overgenerous application can result in burns to skin edges.

Artifactual Abnormalities

Artifactual abnormalities are seen frequently in fetuses following second-trimester termination of pregnancy. Such abnormalities may be the result of trauma during delivery or the effect of subsequent handling of the fetus (Knowles 1986).

The second-trimester abortus has fragile skin and connective tissue so that delivery trauma is easily inflicted. Tearing of skin may occur, and in most sites, particularly limbs, groins, and neck, its traumatic nature will be obvious. If it occurs over the trunk, particularly anteriorly, then confusion with developmental defects of the abdominal wall may arise. Traumatic defects are often in the lower quadrant or flank and have irregular margins within which all layers of the abdominal wall may be identified. There may be associated hemorrhage or necrosis of tissue in the margin of the defect. Intestines and occasionally other organs can prolapse through the defect. Traumatic defects are also seen following spontaneous abortion.

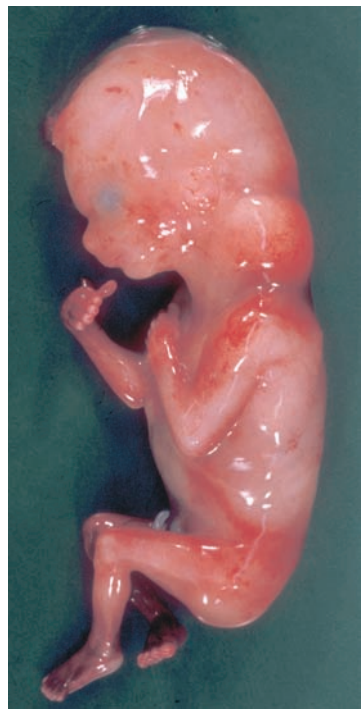


Figure 6.10. Postnuchal soft tissue swelling is the result of brain tissue forced through vertebral foramina.

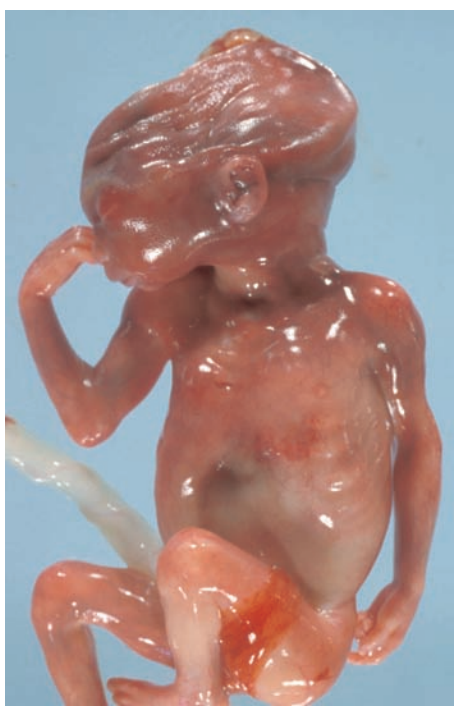


Figure 6.9. Traumatic compression of the head producing an illusion of microcephaly.

Disruption of developmental anomalies may also occur during delivery. The flimsy sacs of meningomyelocele or exomphalos are particularly vulnerable, and vestiges of the sac may be found only after careful scrutiny of the perimeter of the defect or histological examination.

Compression of the head may simulate developmental anomaly (Fig. 6.9) or push cerebral tissue through spinal foramina, producing symmetrical thickening of the neck, resembling cystic hygroma (Fig. 6.10). Incision of the mass reveals semifluid, structureless white-gray brain tissue. Cerebral tissue may be forced down the spinal canal and emerge retroperitoneally, when it may bear more than a passing resemblance to tumor. When forced into the venous system, cardiomegaly, perhaps suggesting malformation, may result (Fig. 6.11).

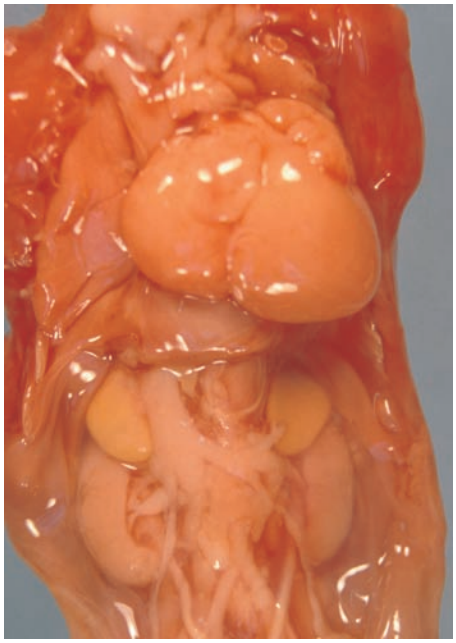


Figure 6.11. Brain tissue forced through great vessels during delivery has produced conspicuous cardiomegaly.

Dislocation of joints can occur during delivery. While dislocations of large joints are easily recognizable, those affecting phalanges may evoke specific dysmorphic features, for example, the hand deformities of trisomy 18 or triploidy.

A long interval between delivery and examination permits the combined effect of gravity and the specimen container to affect fetal contour. Considerable facial asymmetry and distortion can result if the fetus remains in a lateral position for many hours (Fig. 6.12). Such distortion may induce overdiagnosis of dysmorphism or make recognition of dysmorphic features difficult.

Immersion of the fetus in fixative may affect both face and limbs. Soft tissue contraction induces facial deformity and produces contractures of limbs.

Specific Fetal Anomalies

Specific fetal anomalies are discussed within the relevant systems chapters. They are referred to here when particular points need consideration either during fetal examination or where relevant to the differential diagnosis

Hydrocephalus

When hydrocephalus is detected antenatally, it may be an isolated finding or accompanied by other major defects, usually a meningomyelocele. The fetus that terminated because of apparent hydrocephalus must be examined with care for three reasons: first, there are particular pitfalls associated with the ultrasound diagnosis of the condition, so a pregnancy may require repeated monitoring for several weeks before the diagnosis can be made with confidence; second, borderline ventriculomegaly seen on ultrasound scan may be difficult to confirm at autopsy; and third, a precise diagnosis of the cause of the hydrocephalus is important so that a reliable recurrence risk may be derived. Carroll et al. (2000) found that fetal examination was particularly important following imaging diagnosis of Dandy-Walker malformation or its variants.

When there is doubt about the likelihood of confirmation of hydrocephaly by the pathological examination alone, either because of its extent (not marked or particularly gross) or suboptimal fixation, other investigations may complement

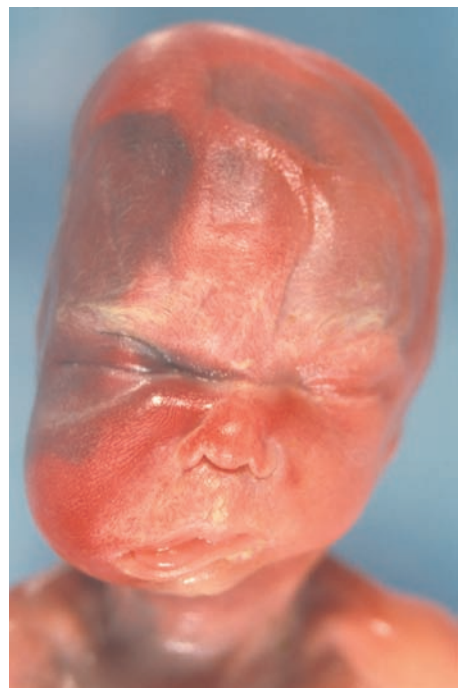


Figure 6.12. Marked facial asymmetry is positional, reflection a long delivery-examination interval.

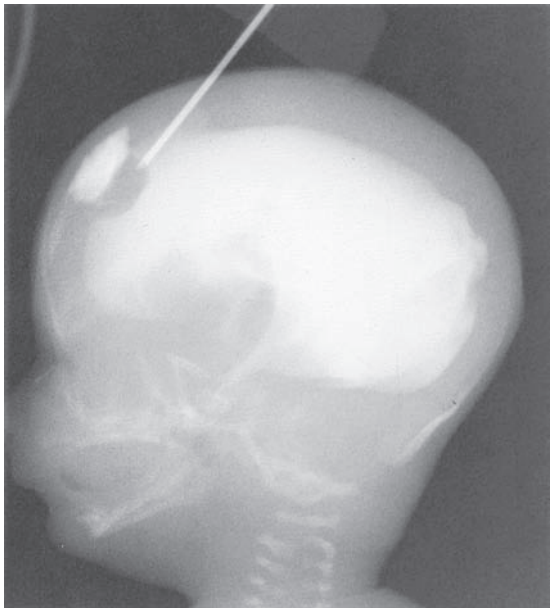
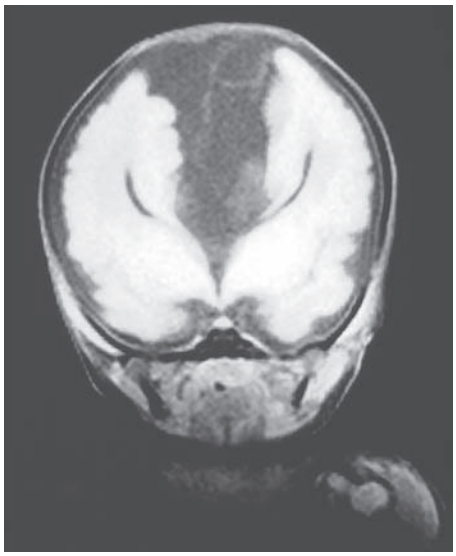


Figure 6.13. Hydrocephaly. Postmortem contrast radiography demonstrates ventriculomegaly.

pathological examination. These include postmortem contrast radiography (Smith et al. 1997) (Fig. 6.13) and MRI (Fig. 6.14).

To achieve these aims, it is essential to fix the brain before examination, optimally by leaving the brain within the cranial cavity. Myelination is poorly advanced at this stage so that attempted removal may defeat all subsequent efforts to elucidate the cause for hydrocephalus.

After 1 to 2 weeks' fixation, the brain will be sufficiently hardened to permit careful handling. It may be removed in the conventional manner (see Chapter 2). Alternatively, individual skull bones may be removed with scissors along a line from the supraorbital ridge to the occiput, and a horizontal cut made through both hemispheres with a thin, broad-bladed knife to remove the rostral portions of the cerebrum (Fig. 6.15) and permit direct comparison of the ventricular contour and ultrasound record. In the second trimester, the cerebral ventricles are much wider, compared with overall cerebral and cortical width, than they are at term, so that an impression of hydrocephalus may be obtained on initial examination of the fetal head. This is when observation of ventricular contour (Fig. 6.15B) may be useful; this contour is lost early in the development of hydrocephalus. Ultrasound studies (Hobbins et al. 1979) have shown that before 24 weeks' gestation hydrocephalus may be marked, while biparietal diameter is within the normal range. This reflects the normally large subdural space at this stage of development, which permits



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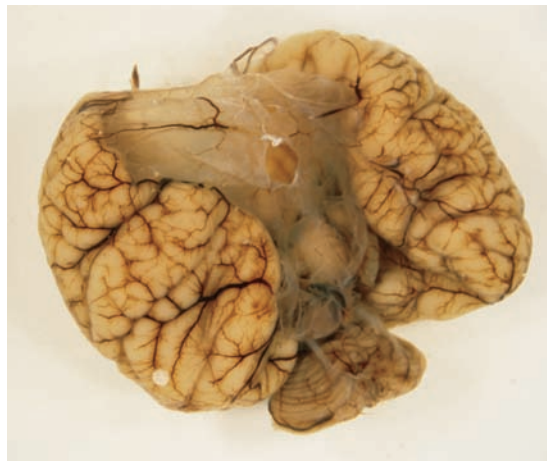


B

Figure 6.14. (A) Postmortem magnetic resonance imaging (MRI) scan. An enormous cyst displaces the cerebral hemispheres laterally. (Dr. M. McPhillips, Edinburgh.) (B) Brain after removal from the cranium and photographed under water. Interpretation of anatomic relationships is made easier by reference to the scan image.

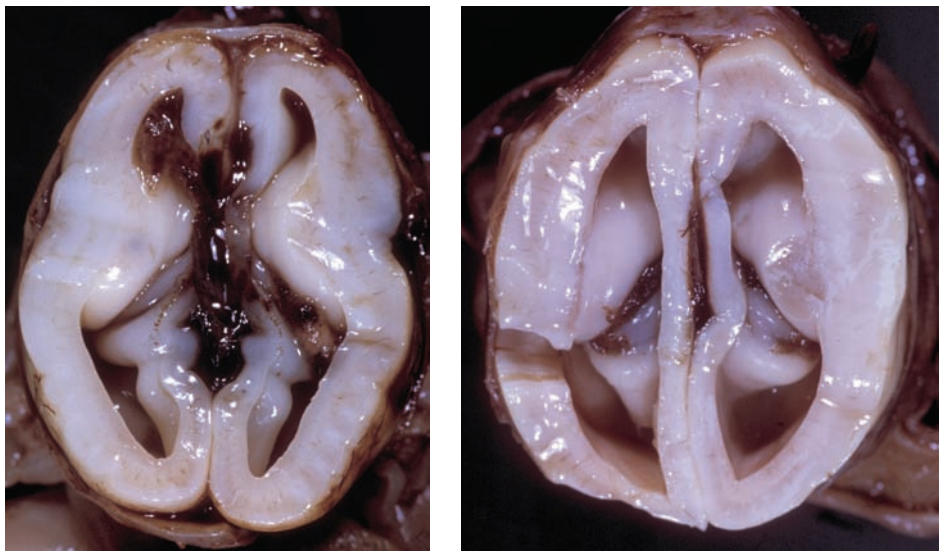


Figure 6.15. Horizontal slice through the fetal brain at 19 to 20 weeks' gestation. (A) Normal brain. Ventricles are relatively large. (B) Hydrocephalus, neural tube defect. There is loss of normal contour, and ventricular dilatation is more marked posteriorly.

a considerable increase in cerebral size before the cerebrum impinges on the inner table of the skull bones.

The cortical mantle and ventricular surface are examined and blocks are taken for histological examination. The critical region, from a diagnostic standpoint, is the pons, midbrain, and medulla. Hydrocephaly is often gross when secondary to aqueduct obstruction (Fig. 6.16A). It is safest to slice the whole region at 2-mm thickness for histological examination, embedding several slices within one block. Obstruction of a previously normal aqueduct as a result of hemorrhagic/ischemic insult or low-grade infection is more commonly encountered than a developmental anomaly in this region. Sections are stained for iron or glial fibrillary acidic protein to better demonstrate gliosis, which may be useful to distinguish between anomalous development and acquired insult. The aqueduct may be obliterated or multichanneled following fetal injury (Fig. 6.16B).

Deformity of the aqueduct, which rarely may be an X-linked recessive condition, has been identified as a cause for hydrocephalus in the second trimester of pregnancy (Harrod et al. 1984). A search for malformations in other systems is important.

Encephalocele

The diagnosis of encephalocele is not usually problematic. It may be covered by normal hair-bearing skin or by membranes (see Fig. 26.5 in Chapter 26). In the former, MSAFP levels are normal, and for this reason often diagnosed later in pregnancy. Occasionally, delivery trauma may produce a spurious appearance of encephalocele when brain is squeezed out of the cranial cavity into the soft tissues of the scalp. No sac lining can be identified, and the cerebral material is without structure. More importantly, there is no defect in the occipital bone (Fig. 6.17).

Encephalocele is the most usual cerebral abnormality seen in Meckel's syndrome (reviewed by Salonen and Paavola 1998). The kidneys and liver should be examined histologically in all fetuses with encephalocele.

Anencephaly

Anencephaly is discussed fully in Chapter 26. It is diagnosed in the first trimester with increasing frequency, and pathological confirmation may be difficult because of disruption of the embryo. While the majority are defects of neural tube

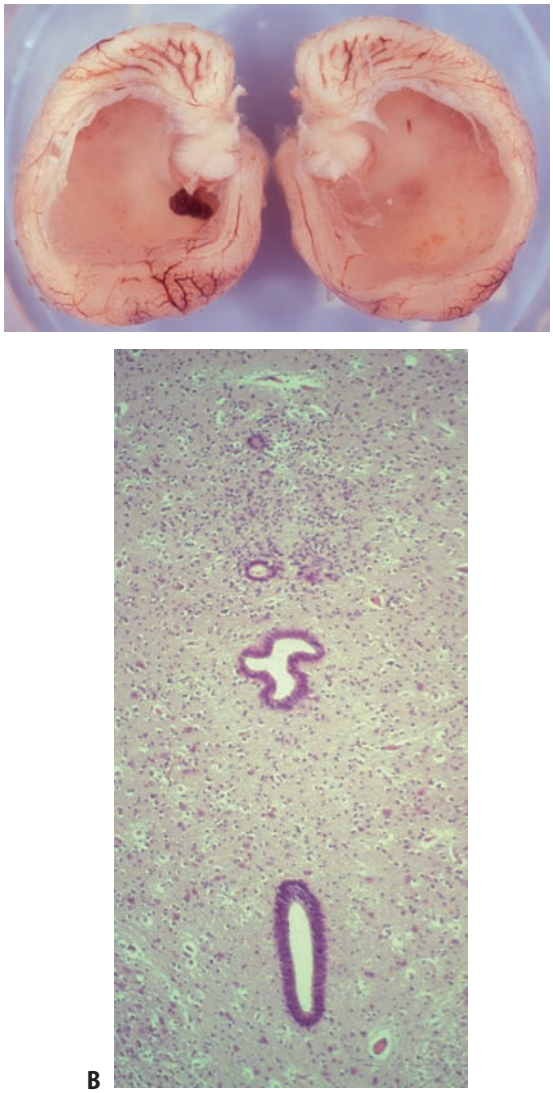


Figure 6.16. (A) Gross hydrocephaly. Brain fixed in situ and supported in water for photography. (B) Histology of the aqueduct, which is seen as several ependymal-lined channels.

closure, and are single defects, a full examination of the fetus is warranted. Trisomy 10q has been demonstrated in recurrent anencephaly with renal abnormalities (Singer et al. 1987). A few cases are the result of amnion rupture sequence. This possibility should be borne in mind when there are other abnormalities of the head, in particular facial clefts not explicable on a developmental basis, or gross facial asymmetry (see Amnion Disruption Sequence p. 155).

Posterior Nuchal Fluid Accumulation/Translucency

Accumulation of fluid in the connective tissues at the back of the neck is a common finding. Its role in screening for fetal abnormality is discussed on Ultrasound Examination: First Trimester p. 132. When it is found at necropsy, full investigation of the fetus, including karyotyping and sampling for molecular investigations, is warranted. Kalousek et al. (1990) found that 85% of fetuses with cystic hygroma have a chromosome abnormality, and Eydoux et al. (1989) found chromosome anomaly in 70% when cystic hygroma was the only malformation diagnosed on scan and in 22% when there were associated malformations. Monosomy X (Turner's syndrome) is the commonest chromosome abnormality, and these fetuses usually have generalized edema (see Fig. 14.3 in Chapter 14) and hypoplasia of the distal aortic arch. Most monosomy X fetuses have one or more cervical ribs (Keeling and Kjaer 1999), a marker of a field defect of paraxial mesenchyme. Trisomies 13, 18, and 21 and some structural chromosome abnormalities may also present with cystic hygroma. Rare single-gene defects such as the lethal multiple pterygium syndrome, some skeletal dysplasias, and Noonan, Robert's and Cumming's syndromes also occur with cystic

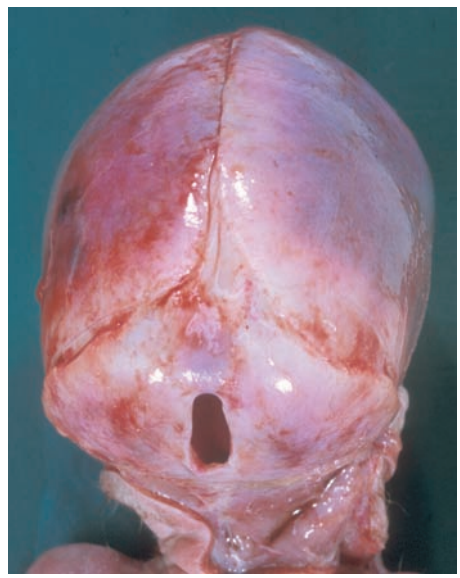


Figure 6.17. Fetus with encephalocele. A defect is present in the occipital bone.

hygroma (Marchese et al. 1985; Boyd et al. 1996). Cowchock et al. (1982) describe postnuchal fluid accumulation accompanied by cleft palate in second-trimester sibling fetuses with normal chromosomes, and Bieber et al. (1979) reported cystic hygroma in siblings with ascites and edema. A dominant form has been reported (Graham and Smith 1981), as well as an entirely normal liveborn after diagnosis of cystic hygroma in the second trimester (Macken et al. 1989).

Chitayat et al. (1989) studied the structure and number of lymph vessels in 12 spontaneous abortions of fetuses with posterior cystic hygroma and generalized edema of variable etiology and compared them to five therapeutically aborted apparently normal fetuses. They found that in the non-Turner's fetus there was an increase in number and dilatation of lymphatic vessels, whereas the Turner's fetus had no recognizable lymph vessels in the edematous cutaneous tissues of the limbs and sparse dilated vessels in the wall of the cystic hygroma and lungs.

Cervical teratoma may be mistakenly diagnosed on ultrasound as cystic hygroma but it causes little diagnostic problem for the pathologist. Postnuchal fluid accumulation has been inadvertently diagnosed as occipital encephalocele (Nevin et al. 1983) and must also be distinguished from cervical meningocele. Minor degrees of postnuchal fluid accumulation are seen in spontaneous abortion, both fresh and macerated, and in the presence of a variety of apparently unrelated abnormalities.

Abdominal Wall Defects

Anterior abdominal wall defects are described in Chapter 18. Attention may be drawn to their presence during the second trimester because of raised MSAFP level, or an abnormal ultrasound scan.

It is usually possible to distinguish among the three commonest types of defect in utero, and the distinction should most certainly be made postnatally.

Omphalocele and gastroschisis are distinct abnormalities. The etiology of each is unclear and there is some controversy concerning pathogenesis (Kluth and Lambrecht 1996).

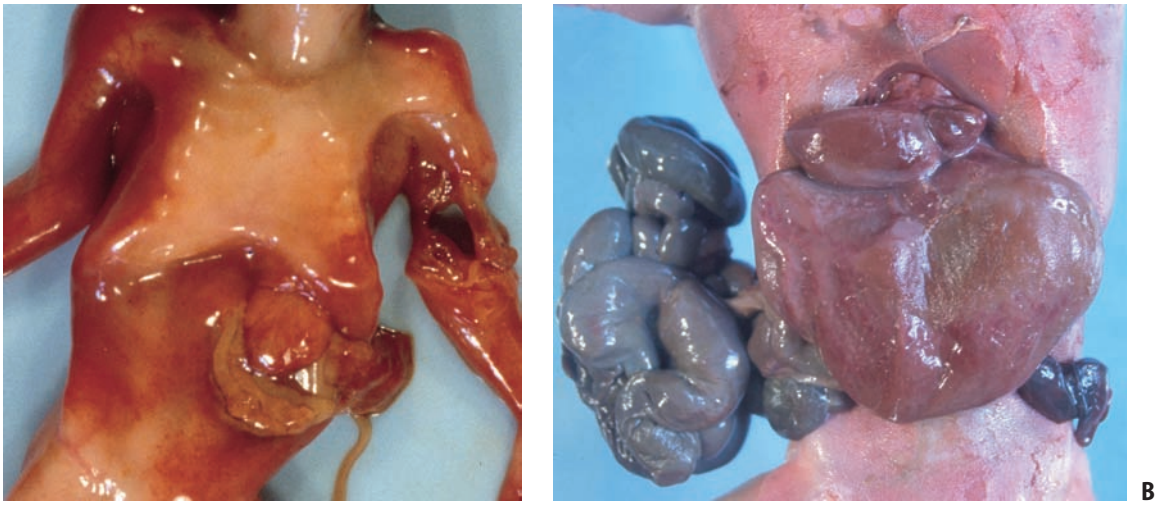
Omphalocele is a midline defect with herniation of the abdominal contents into the base of the umbilical cord. Abdominal contents are enclosed in a membranous sac composed of amnion and peritoneum, and the umbilical cord arises from the apex of the sac (see Figs. 18.25 and 18.26 in Chapter 18). Associated major defects are frequent. Boyd et al. (1998) found that 54% of omphaloceles were accompanied by other defects compared with 5% of those with gastroschisis. Overall 29% of omphaloceles had an abnormal karyotype, and of those with another abnormality identified on scan (excluding four cases with no karyotype performed), 54% had abnormal karyotype. Six of 27 cases of prenatally suspected isolated omphalocele were subsequently diagnosed as having BWS. Mann et al. (1984) found that 53% of their cases had other major defects and one third of those karyotyped had autosomal trisomy. Nicolaides et al. (1986) found chromosome abnormalities in two thirds of fetuses karyotyped because of prenatal diagnosis of exomphalos, but referral patterns may have introduced bias toward cases with multiple anomalies. Torfs et al. (1990) reported that one third of 81 cases of omphalocele had recognizable syndromes, particularly trisomies 13 and 18 and BWS.

Gastroschisis is a (usually right-sided) paraumbilical defect with evisceration of abdominal contents directly into the amniotic cavity (see Fig. 18.27 in Chapter 18). It is associated with low maternal age and low birth weight and may have a vascular cause (Hoyme et al. 1981).

The incidence of associated defects is lower in gastroschisis (5–24%) than with omphalocele (30–75%) (Torfs et al. 1990; Boyd et al. 1998). Many of the defects are local, particularly intestinal atresia, thought to result from volvulus of abnormally mobile bowel loops. Mann et al. (1984) observed neural tube defect in two of their 16 cases.

The limb defects and relative fetal immobility of body stalk defects (see Fig. 18.28 in Chapter 18) mean that they are usually identified correctly in utero. Complex defects involving the thorax and upper abdomen are much less common. Prolapse of organs, including the heart, into the amniotic cavity (Fig. 6.18) reduces body cavity volume. Structural cardiac defects may be present.

When pathological examination is performed, a search for minor dysmorphic features as well as major defects should be undertaken, and blood



A B

Figure 6.18. (A) Upper abdominal wall defect. The heart, intestine and spleen protrude through the defect. (B) Extensive upper abdominal defect with thoracic extension. The heart and most abdominal viscera are exteriorized.

and tissue should be sent for chromosome analysis on the slightest suspicion of dysmorphism.

Cystic Kidneys

Cystic enlargement of the fetal kidney may be discovered during routine ultrasound examination, or attention may be drawn to the possibility of renal malformation by oligohydramnios, during a deliberate search for cystic renal disease, or during the investigation of certain metabolic abnormalities in women at risk. Cystic kidneys may be identified because of raised MSAFP level caused by increased concentration of AFP because of oligohydramnios or the presence of an associated anomaly.

It is important to distinguish among the different types of renal cystic disease so that accurate risks of recurrence are given to parents and appropriate investigations undertaken in subsequent pregnancies. Renal cystic disease is discussed in Chapter 22. Some diseases result from single-gene defects, which predominantly affect the kidney, while others are manifestations of a genetic metabolic disorder, such as cerebrohepatorenal syndrome (Powers et al. 1985) and glutaric acidemia type II (Boué et al. 1984) (Fig. 6.19) and other syndromes with diverse systematic defects. Renomegaly is unusual before 19 weeks' gesta-

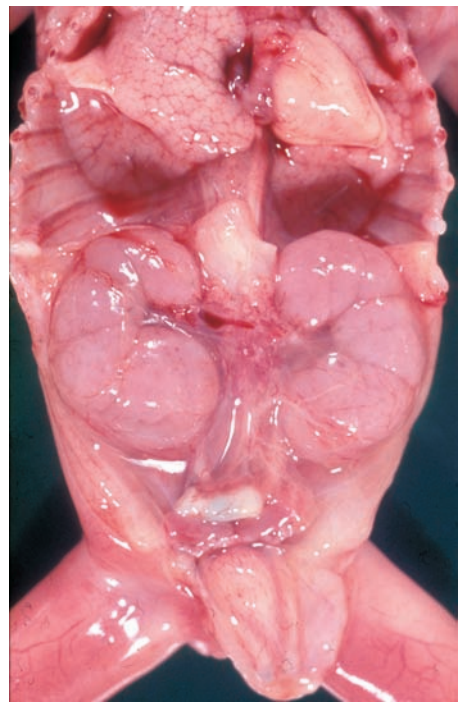


Figure 6.19. Glutaric acidemia type II. Renomegaly was apparent on pre-amniocentesis scan in an at-risk fetus. Renomegaly is not as marked as in Fig. 6.20.

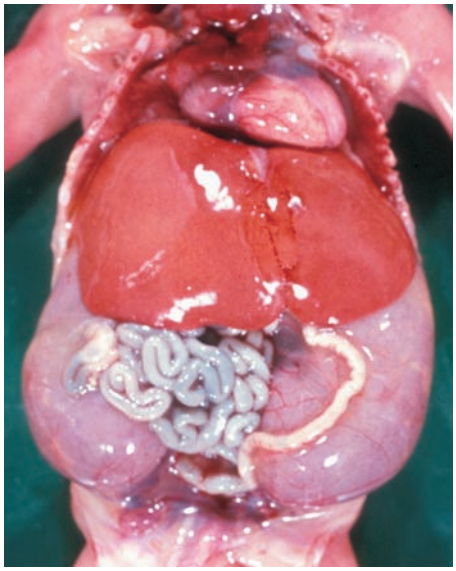


Figure 6.20. Autosomal recessive polycystic kidney disease (ARPKD). Enormous renomegaly is present by 19 weeks' gestation.

tion, and the different types of cystic dysplasia may be distinguished histologically toward the end of the second trimester. Both the fetus with autosomal recessive polycystic kidney disease (ARPKD) (Fig. 6.20) and that with Meckel syndrome (Fig. 6.21) develop symmetrical reniform enlargement from around 18 weeks' gestation. In both cases, small cysts can be seen through the renal capsule. In Meckel's syndrome, the degree of renal enlargement is variable. Rapola (1989) describes renal weights between 9 and 36 times normal for gestation. He also draws attention to the prominent and histologically normal nephrogenic zone present at this stage. The bladder is often rudimentary as the volume of urine is very small. It is unusual to detect liver cysts during dissection in the 18- to 20-week fetus, despite liver enlargement, but a ductal plate abnormality may be apparent histologically.

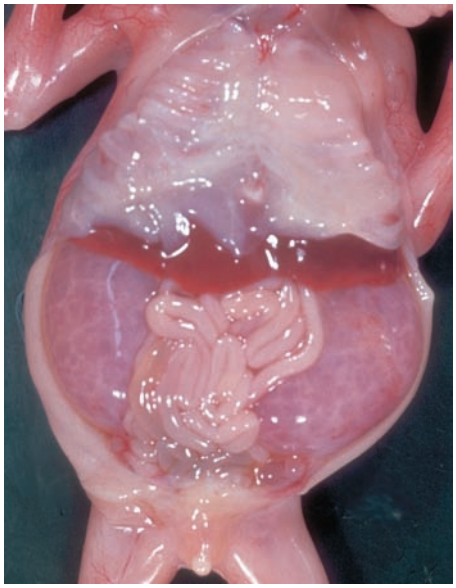


Figure 6.21. Meckel's syndrome. Symmetrical renomegaly at 18 weeks' gestation.

Cystic renal dysplasia presents with oligohydramnios when it is bilateral or accompanied by agenesis of the contralateral kidney, which often makes detailed ultrasound examination difficult. This type of renal abnormality may be seen both with and without lower urinary tract obstruction (see Fig. 22.3 in Chapter 22). It may be the first intimation of a recognized syndrome, for example Fraser's syndrome (Boyd et al. 1988) or autosomal trisomy. Detailed examination of the fetus may elicit a pattern of dysmorphic features to back up this suspicion.

Chromosome Anomalies

The most commonly encountered chromosome anomalies in fetuses from deliberately terminated pregnancies are trisomies 21 and 18 and monosomy X (Turner's syndrome). When pregnancy is being terminated for chromosome anomaly, confirmation of fetal karyotype is an important part of fetal assessment and can be done by FISH or culture and karyotype on fetal tissue samples.

When trisomy 18 is present, characteristic dysmorphic features are always identifiable. A globular head with hypertelorism, broad nose, and micrognathia is usual. Overlapping of fingers (Fig. 6.22) is easily distinguished and almost always present. Flexion deformities of lower limbs with abnormalities of the feet are usual. Major anomalies of viscera are frequently found in trisomies 13 and 18 (Isaksen et al. 2000). Table 6.6 shows the dysmorphic features and visceral anomalies found in locally examined material.

The fetus with trisomy 21 does not have such easily recognizable abnormalities in the second trimester. The typical facies seen in the infant with

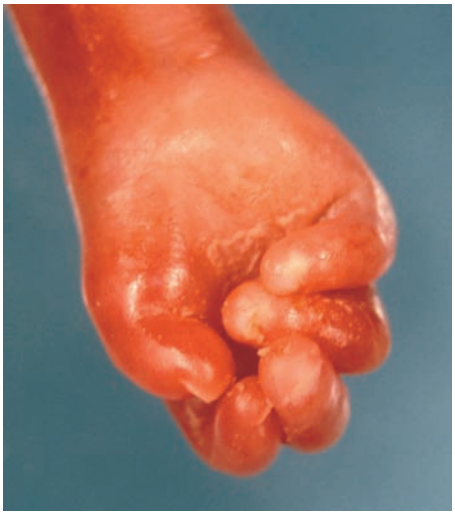


Figure 6.22. Trisomy 18. Overfolding of fingers is apparent at 19 weeks' gestation.

Table 6.6. External malformations and visceral anomalies in 43 fetuses with trisomy 18 karyotype; all fetuses had two or more dysmorphic features

Cleft lip and palate	5
Radial aplasia	5
Exomphalos	9
Incomplete intestinal rotation	14
Diaphragmatic eventration/hernia	3
Tracheoesophageal fistula	4
Heterotopic pancreas	3
Intestinal duplication cyst	2
Cerebral anomaly	8
Meningomyelocele	9
Renal tract anomaly	17
Ventricular septal defect	22
Double-outlet right ventricle	5
Bicuspid pulmonary valve	4
Anomalous aortic arch branching	2
Hypoplastic left heart	1

Down syndrome has not developed. While some fetuses with trisomy 21 have readily identifiable facial dysmorphism in the second trimester, it is neither so marked nor so consistent (Fig. 6.23) as that seen in trisomy 18; indeed, some fetuses appear normal even to an experienced observer. Bilateral transverse palmar creases are present in one half to two thirds of cases, and a single palmar crease in a few of the remainder. A prominent space (sandal gap) between first and second toes is frequently present. In some fetuses, short digits camptodactyly of fifth fingers and broad hands

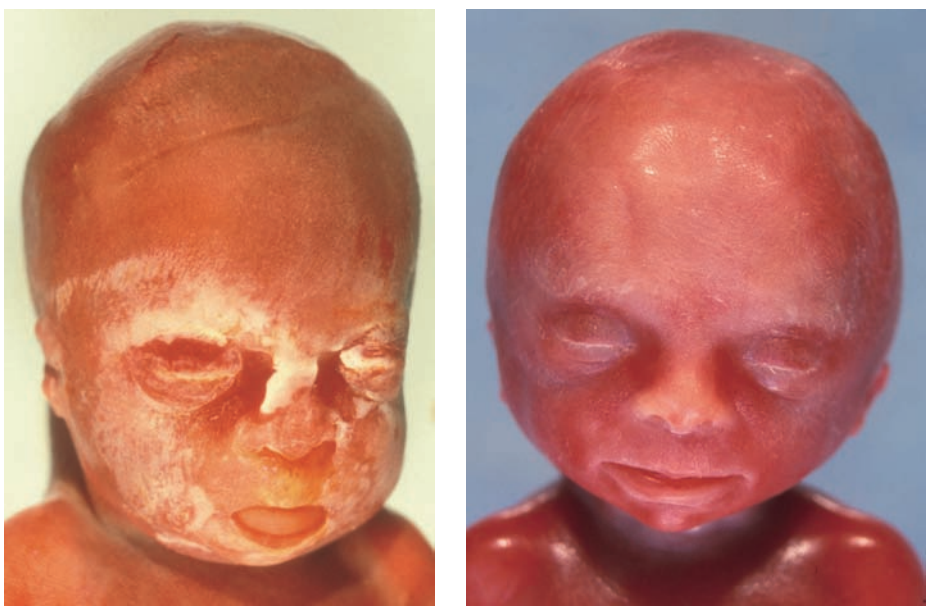


Figure 6.23. Trisomy 21. (A) Marked facial dysmorphism with prominent brow. Small nose and protruding tongue is easily recognized. (B) Fetus is dysmorphic, but not readily diagnosed as trisomy 21.

may be apparent. Visceral anomalies (Table 6.7) are not seen as frequently as in trisomy 18 and are usually single (Fig. 6.24; see also Fig. 21.8 in Chapter 21).

Monosomy X is sometimes picked up by chance because of karyotyping performed for maternal age but more commonly is detected following identification of posterior nuchal fluid with or without hydrops during ultrasound examination in the first or second trimester. Anomalies of the aortic arch, particularly hypoplasia of its third part (see Fig. 14.5 in Chapter 14) and aberrant origin of the right subchorionic artery, are the most frequently identified abnormalities. A perimembranous ventricular septal defect and cervical ribs may be present. The ovaries are normal at this stage of development.

Dysmorphic features found in other chromosomic anomalies (Fig. 6.25) are detailed by Jones (2005). The triploid fetus is often severely growth restricted, major malformations are common, dysmorphisms are almost universal (Mittal et al. 1998), and intrauterine death in the second or third trimester is usual. Common malformations include meningomyelocele, hydrocephaly, cardiac defects, and cleft lip and palate.

Table 6.7. Dysmorphic features and visceral malformation in 60 fetuses with trisomy 21 karyotype

Coarse features	43
Low set, simple ears	31
Nuchal edema	19
Fetal hydrops	7
Short neck	9
Transverse palmar crease	
Bilateral	21
Unilateral	15
Clinodactyly	10
Short fingers	8
Abnormal palmar crease	4
Syndactyly	3
Bifid thumb	1
Vertical plantar crease	21
Prominent heels	3
Talipes	1
Atrioventricular canal defect	8
Ventricular septal defect	7
Atrial septal defect	3
Pulmonary valve atresia	3
Right-sided aortic arch	1
Duodenal atresia	2
Duodenal duplication, ectopic pancreas	1
Incomplete rotation bowel	3
Imperforate anus	1
Hydrocephalus	2
Microcephaly	1
Abnormal lung lobation	2
Cystic kidney	1
Hydronephrosis, hypersegmentation	2

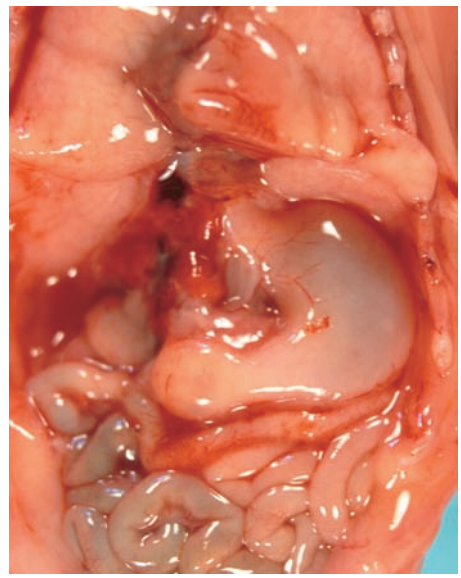


Figure 6.24. Trisomy 21, duodenal atresia. Dilatation of the duodenum is apparent beyond the pylorus.

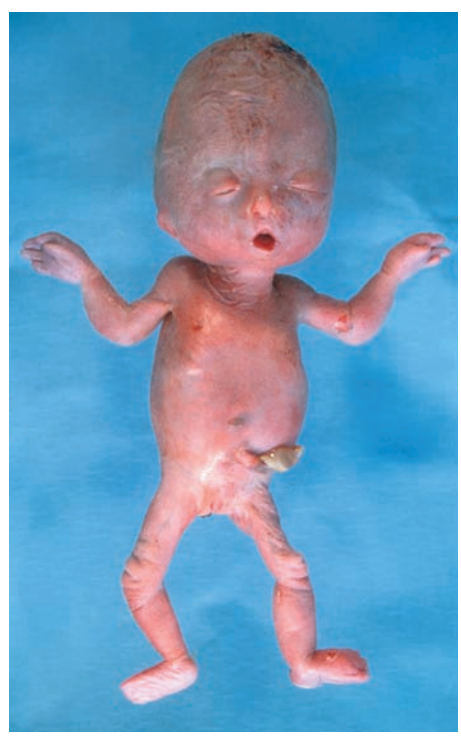


Figure 6.25. Triploidy. Growth-restricted fetus with a large head and facial and acral dysmorphism.

Amnion Disruption Sequence

Rupture of the amnion in the first half of pregnancy gives rise to a range of anomalies, which seem to be related to the time of amnion rupture (Higginbottom et al. 1979). It is important to recognize the results of amnion rupture, which may include disruption and deformity of major proportions (Fig. 6.26), because the recurrence risk in most cases is negligible. Amnion rupture sequence is misdiagnosed when attention is paid only to the most striking anomaly, which may resemble a defect with known recurrence risk, while atypical features and associated anomalies are ignored (Seeds et al. 1982).

A spectrum of defects is described. The important observations that emerge are as follows:

- There is a striking asymmetry of defects.
- A combination of defects is found that may or may not be explained by our knowledge of normal development.
- No two individuals have the same combination of anomalies.

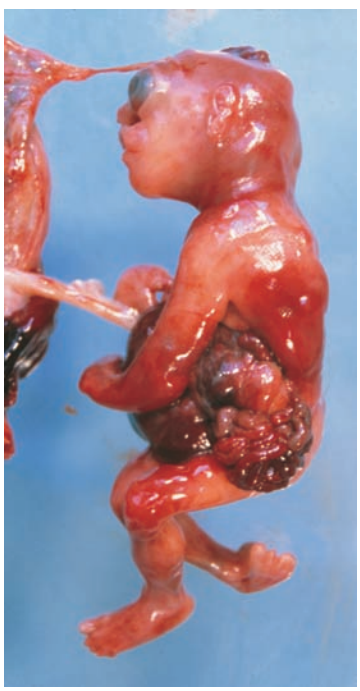


Figure 6.26. Amnion disruption. There is anencephaly, and a tissue band runs from the supraorbital ridge to the placenta.

While it is generally accepted that amnion rupture is a common factor in the development of this group of defects, the cause of such rupture is not clear. Physical trauma in early pregnancy has been recognized in a very few cases (Jones 2005). Sequelae of amnion rupture are described in infants with connective tissue disorders such as Ehlers–Danlos syndrome and severe osteogenesis imperfecta (Young et al. 1985). In these disorders, defective collagen probably reduces the load-bearing capacity of the placental membranes. A small excess of summer conceptions and continued ingestion of oral contraceptives in early pregnancy are also described (Ossipoff and Hall 1977). Amniotic bands are described following amniocentesis (Rehder and Weitzel 1978). Pregnancy complications among locally examined cases include those due to maternal drug abuse, anorexia nervosa, and in vitro fertilization (IVF). Infection does not seem to be a factor in the etiology of amnion rupture. MacIntyre et al. (1995) observed a high incidence of limb abnormalities and a lower level of palatal defects in a mouse model following amniotic sac puncture at day 13 or 14 of pregnancy, lending support to the importance of amnion insult.

A human homologue of the mouse disorganization gene (*Ds*) has been proposed as a cause of at least some cases of amnion disruption (Donnai and Winter 1989; Crosby et al. 1993). This is a semidominant gene, and in the mouse 72% of heterozygotes have anomalies. It might explain those visceral defects unrelated to musculocutaneous disruption and the infrequent familial cases.

Amnion rupture sequence with major defects is frequently diagnosed because of raised MSAFP levels, and some are identified by ultrasound examination. The range of anomalies resulting from amnion rupture is shown in Table 6.8. Observed anomalies arise as a result of a combination of mechanisms causing physical interruption of normal development, tethering, compression, and injury to previously formed structures. Major defects arise early in gestation and are often accompanied by reduction of fetal movements because of firm attachment of fetal parts to the chorion, which results in a very short umbilical cord. Visceral defects are infrequent and usually only found in relation to disruption of the fetal surface. Bamforth (1993) draws atten-

Table 6.8. Types of anomaly seen in amnion rupture sequence

Time of rupture	Craniofacial	Trunk	Limbs	Placenta
<4 weeks	Anencephaly Inappropriate facial cleft Proboscis, eye defects Encephalocele		Absent limb	Adherence of head to chorion
<6 weeks	Cleft lip	Thoracic wall defect Abdominal wall defect Severe scoliosis	Syndactyly Limb reduction	Abdominal wall attached to chorion Short or absent umbilical cord
<12 weeks	Cleft palate Ear deformity Craniostenosis	Omphalocele	Amputation of digits/part limb Limb hypoplasia Asymmetrical limb size	Short umbilical cord Amnion "strings" or bands
>12 weeks			Compression deformities	Oligohydramnios

tion to "regionalization" of visceral anomalies, particularly the excess of renal anomalies seen with lower limb deformity. The possibility of a preexisting defect of embryogenesis with secondary amnion adhesion/disruption should be borne in mind (Keeling and Kjaer 1994).

When pregnancy terminates in the second trimester, intact bands of amnion may be identified running between the defect and the chorionic sac. In the more mature fetus, continuity is usually lost, particularly if the fetus is stillborn (Fig. 6.27), and a few degenerate threads remain, although a complete encircling band may be seen applied to a limb constriction defect. Should the fetus have emerged from the amniotic sac into a secondary space between the placental membranes, then the amniotic sac may persist as a frill around the cord insertion, occasionally a small amniotic sac may be identified.

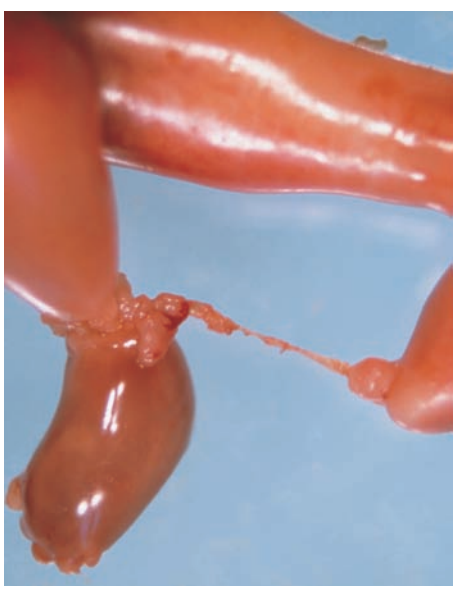


Figure 6.27. A band of amnion runs between the left ankle and right first toe. The left foot is swollen and the distal phalanges are lost.

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7
Genetic Metabolic Disease

David R. FitzPatrick

The terms *inborn errors of metabolism, genetic metabolic disease, disorders of intermediary metabolism,* and *inherited metabolic disease* are used interchangeably. For the sake of clarity, inborn errors of metabolism (IEMs) is used in this chapter. The common feature of these disorders is a genetically determined interruption in one (or several related) metabolic pathway. This results in clinical symptoms caused by deficiency of the pathway product or toxicity resulting from the accumulation of an intermediary compound (Fig. 7.1). Inborn errors of metabolism are mostly genetically recessive disorders with clinical symptoms rare in heterozygous individuals. The molecular pathology of IEMs usually involve homozygous (autosomal) or hemizygous (X-linked) loss of function mutations in genes encoding proteins with a single enzymatic function. This chapter catalogues the main modes of presentation of genetic metabolic disease during fetal and neonatal life and currently available laboratory diagnostic tools. It should be noted that new phenotypes and diagnostic techniques are continually evolving and online services, such as the On-Line Mendelian Inheritance in Man (OMIM) (see Appendix 7.1), can be a very helpful adjunct to hardcopy reference texts.

General Principles of Inborn Errors of Metabolism

Phenylketonuria (PKU) has become the paradigm of successful diagnosis and treatment of IEM. It is caused by homozygous loss-of-function mutations in the gene encoding a liver-specific enzyme involved in amino acid metabolism, phenylalanine hydroxylase (PAH), on chromosome 12. The clinical features of PKU are caused by both the deficiency of the enzymatic reaction product (tyrosine) and accumulation of the reaction precursor (phenylalanine). In untreated PKU, a deficiency of tyrosine, a precursor of melanin, causes general hypopigmentation (the blue eyes and fair hair seen in photographs of affected individuals in historical textbooks). High tissue phenylalanine levels result in mental retardation, although the pathophysiology of this effect is unclear. The accumulated phenylalanine undergoes alternative metabolism via enzymatic and nonenzymatic reactions, which allows the detection of metabolites from these alternative pathways, such as phenylacetate, in body fluids.

In genetic terms, most forms of IEM involve loss of function mutations in a gene encoding an enzyme that catalyses a particular reaction (e.g., PAH) in a biosynthetic or catabolic pathway. Interruptions in biochemical pathways may also result from mutations affecting the bioavailability of a enzymatic cofactor (e.g., disordered tetrahydrobiopterin synthesis, a cofactor of PAH, in a severe variant of PKU known as malignant PKU) and disordered transport of the enzyme across cellular membranes (e.g., the β-oxidation defect in Zellweger syndrome). In both these situations it is likely that more than one enzymatic process will be affected.

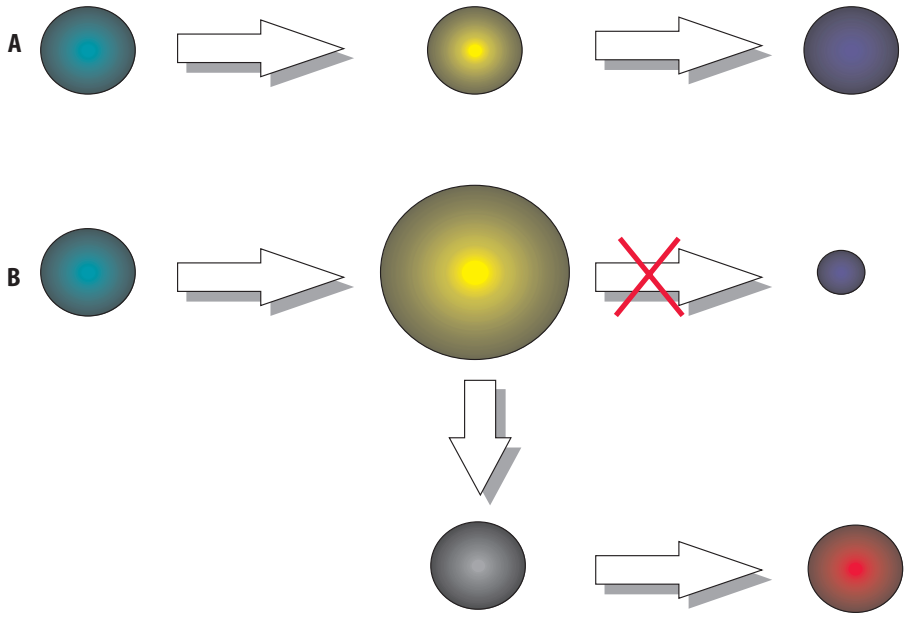

FIGURE 7.1. Diagrammatic representation of the biochemical effects of inborn errors of metabolism (IEMs). (A) Normal metabolic pathway in which the substrate (green sphere) is converted to the product (blue sphere) via two separate enzymatic reactions (arrows) producing an intermediate substance (yellow sphere). (B) If the second enzymatic reaction is genetically deficient, this will result in deficiency of the product and accumulation of the intermediary substance. This accumulation may cause toxic metabolites of the accumulated substance to form (gray and red spheres).

Laboratory Investigations in Genetic Metabolic Disease

Metabolite Analysis

Many IEMs were originally identified using simple chemical tests to detect the presence of an abnormal metabolite. Such tests include the following:

- Ferric chloride test, which detects phenylalanine metabolites in urine
- Reducing sugars in the urine (Clinitest, Ames), which detects galactosemia, hereditary fructose intolerance, and diabetes mellitus
- Acetone (Acetest, Ames), which is positive in some organic acidemias and glycogenoses
- Keto acids (dinitrophenylhydrazine), which are useful in diagnosing maple syrup urine disease (see Aminoacidopathies, below)
- Sulfitest (Merck), which is used for diagnosis of molybdenum cofactor deficiency

These tests are relatively nonspecific and may give false-positive results with commonly used drugs. The more recent ability to accurately measure glucose, lactate, and ammonia in plasma samples has been enormously beneficial in the identification of infants with IEM, although they seldom enable the investigating clinician to make a specific diagnosis without further testing. The development of increasingly sophisticated detection systems has enabled the identification of an increasing number of intermediate metabolites, and such analyses remain the most important step in the diagnosis of IEM in the neonatal period.

Amino Acids

Inborn errors of metabolism that interrupt the normal catabolism of amino acids in the very early stages of the pathway may be diagnosed by examination of the amino acid content of various body fluids. Most of the known disorders of amino acid metabolism were identified using semiquantitative chromatographic techniques (paper and/or thin-layer chromatography). These

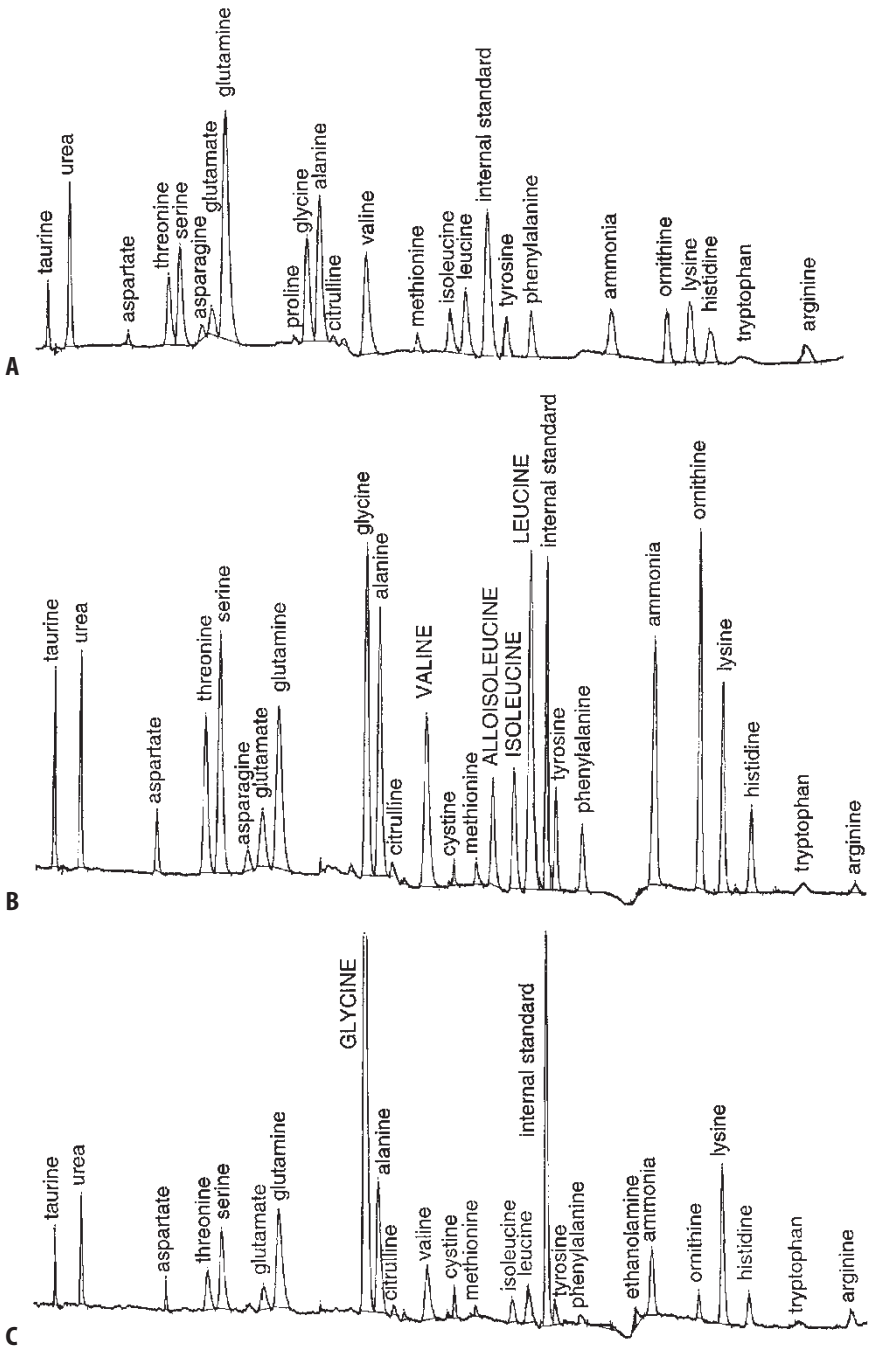

Figure 7.2. Amino acid chromatography. (A) Normal plasma amino acid profile shown by quantitative chromatography. (B) Abnormal plasma profile showing a marked increase in the levels of the branch chain amino acids in a treated patient with maple syrup urine disease. Leucine 606 μmol/L (normal 60–230 μmol/L), isoleucine 187 μmol/L (normal 20–100 μmol/L), valine 331 μmol/L (normal 100–330 μmol/L), and alloisoleucine 168 μmol/L (normal <5 μmol/L). (C) Nonketotic hyperglycinemia; plasma from a boy aged 16 days on sodium benzoate. Plasma glycine 1048 μmol/L (normal 100–390 μmol/L).

were replaced in most centers by quantitative analysis of amino acids using ion exchange chromatography, high-performance liquid chromatography (HPLC), gas chromatography, or most recently tandem mass spectrometry. These techniques are sensitive and specific and facilitate the separation of all physiological amino acid into individual peaks (Fig. 7.2). In postnatal life, amino acids should be analyzed from both blood and urine (and indeed other body fluids) as some amino acids (e.g., arginosuccinic acid) are cleared very rapidly from the blood but are present in high levels in the urine. It should also be appreciated that disorders of amino acid metabolism may occasionally present as highly tissue-specific diseases such as cerebral nonketotic hyperglycinemia (Applegarth and Toone 2001; Hoover-Fong et al. 2004).

Organic and Fatty Acids

Inborn errors of metabolism that interrupt amino acid metabolism at a latter stage in the pathway result in the accumulation of acyl–coenzyme A (CoA) compounds. These compounds may undergo various reactions in the cell, and the product of these reactions are known as organic acids. Organic acids are not detected using amino acid analysis. Gas chromatography and mass spectrometry (GC-MS), tandem mass spectrometry (TMS), and nuclear magnetic resonance (NMR) are the main diagnostic tools used for detecting organic acids in urine, blood, and cerebrospinal fluid (CSF) (Fig. 7.3). These are all very sensitive diagnostic instruments, and some IEMs may result in a bewilderingly complicated pattern of abnormal metabolites. Fortunately, detailed metabolite mass spectral libraries have been developed by specialist laboratories to aid diagnosis of many IEMs. The diagnosis of IEMs that are disturbing normal fatty acid oxidation has been revolutionized following the introduction of tandem mass spectrometry identification of diagnostic plasma acyl-carnitine profiles. Tandem mass spectrometry is particularly useful in the analysis of cases of sudden infant death (Olpin 2004). It has been the history of IEM research that each new technology will allow the identification of new classes of disease and a wider spectrum of clinical presentation of known disorders. It is, therefore, important to cast the diagnostic net wide in the early stages of clinical analysis as the spectrum of diseases associated with IEM is growing each year.

Neurotransmitter Analysis and In Vivo Neurometabolic Techniques

It is becoming clear that infantile-onset seizure disorders and early-onset dystonia are commonly associated with neurometabolic disorders that often require specific investigations for analysis. In postnatal life, chromatographic analysis of CSF is useful if both collection and analysis are carefully controlled (Hoffmann et al. 1998; Surtees 1999; Assmann et al. 2003). It is unlikely that such investigations will yield useful information postmortem. Magnetic resonance spectroscopy (MRS) is a noninvasive neuroimaging technique that can identify metabolites in vivo. It has been used to identify a new class of IEMs called disorders of creatine synthesis and transport (Stromberger et al. 2003) and has great potential as a diagnostic tool.

Enzyme Assay

A specific IEM is often initially diagnosed by identification of an accumulated metabolite. This usually requires confirmation by direct enzymatic assay in cells or tissues such as plasma or serum (e.g., Tay-Sachs disease), red blood cells (e.g., galactosemia), leukocytes (e.g., many lysosomal enzymes), cultured skin fibroblasts (e.g., pyruvate dehydrogenase), liver (carbamyl phosphate synthetase), intestinal mucosa (e.g., ornithine transcarbamylase), and muscle (e.g., respiratory chain disorders). The confirmation of specific enzyme defects may be particularly useful in offering prenatal diagnosis for future pregnancies.

Histology and Immunocytochemistry

The abnormal accumulation of specific metabolites may be recognized by histological or histochemical analyses. This is particularly useful in the diagnosis of glycogen storage diseases, in which deposition of glycogen in affected tissues is often seen and in lysosomal storage diseases where intracellular vacuolation is due to the deposition of various lipids and glycoproteins

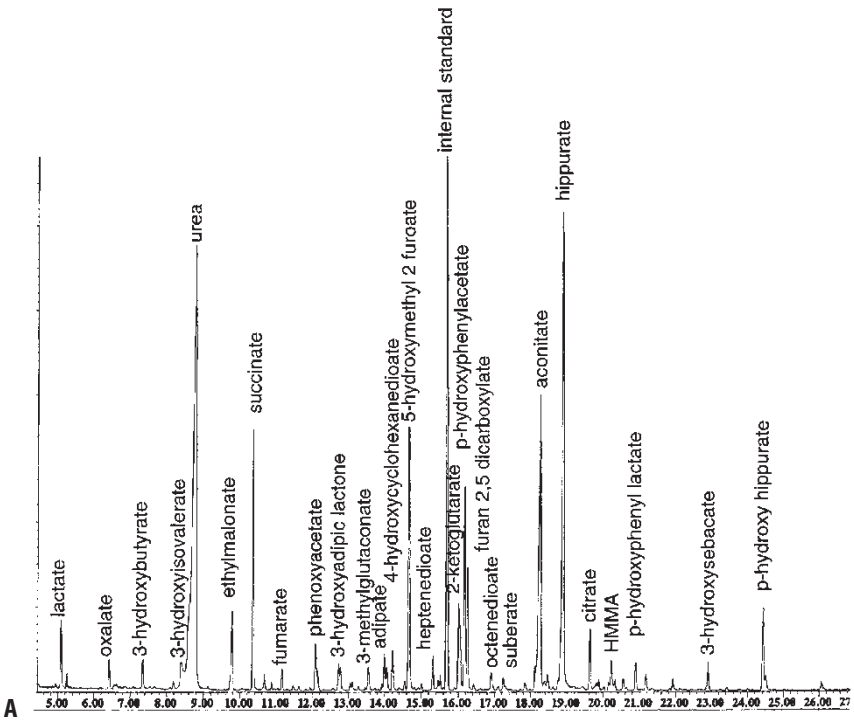

FIGURE 7.3. Gas chromatography and mass spectroscopy (CG/MS). (A) Total ion chromatogram of organic acids in normal urine. (B) Mass spectrum of individual peaks and its library match.

in abnormal organelles (Fig. 7.4). Immunocytochemistry is widely used in modern pathology and may be helpful in defining genetically heterogeneous IEMs such as mitochondrial myopathies secondary to oxidative phosphorylation defects. Immunological analysis of IEMs may yield false-negative results due to the presence of nonfunctional but immunoreactive mutant protein within the tissue.

DNA Analysis

Polymerase chain reaction (PCR) amplification of preselected segments of genetic material from genomic DNA enables the detection of specific pathogenic mutations in DNA samples. In some IEMs a small number of mutations account for a large percentage of mutant chromosomes, facilitating mutation analysis to become the first-line diagnostic test. This is true of medium-chain acyl-CoA dehydrogenase deficiency where >90% of cases are homozygous for a single point mutation in the *MCAD* gene, which can be easily detected by a PCR assay (Fig. 7.5). It is likely that as the genes encoding all enzymes are cloned and causative mutations recognized, DNA diagnosis will be more widely used in the investigation of IEM in the future.

Clinical Presentations of Inborn Errors of Metabolism in Fetal Life

It is axiomatic that the most important step in diagnosing any genetic biochemical disorder is the clinician's accepting that such a diagnosis should be entertained. This is particularly true in this section since the recognition of genetic biochemical disease presenting in fetal life is relatively recent. The main modes of presentation are the following:

- Nonimmune hydrops fetalis
- Maternal intoxication

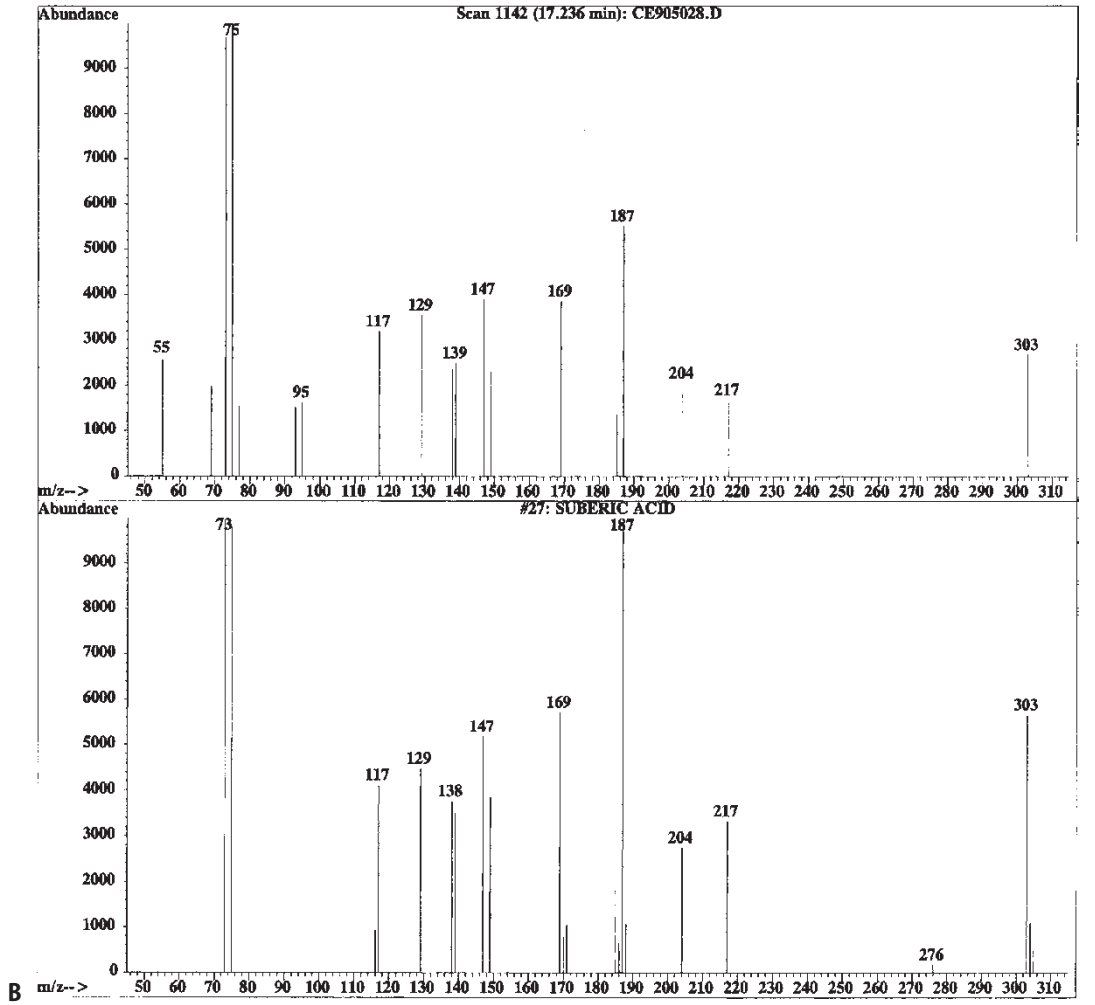

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FIGURE 7.3. *Continued*

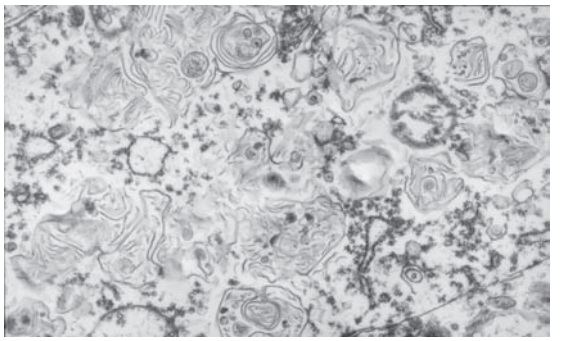

FIGURE 7.4. Electron microscopy analysis of brain tissue in a child with GM_1 gangliosidosis showing intracellular vacuolation is due to the deposition of various lipids and glycoproteins in abnormal organelles.

- Central nervous system (CNS) malformations with or without other birth defects (see Clinical Presentations of Inborn Errors of Metabolism in the Neonatal Period, below)
- Prenatal diagnosis on the basis of DNA, enzymatic or metabolite analysis (see Laboratory Investigations in Genetic Metabolic Disease, above)

Hydrops Fetalis

Nonimmune hydrops (NIH), covered in detail in Chapter 14, shows extreme etiological heterogeneity. The IEMs are rare causes of NIH, accounting for 1% to 2% of all cases (Van Maldergem

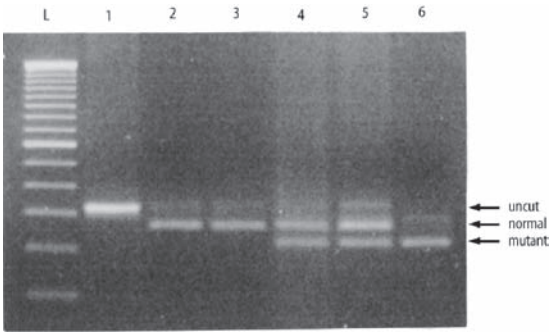

FIGURE 7.5. Polymerase chain reaction (PCR) analysis of mutations in the *MCAD* gene. Agarose gel electrophoresis of amplified DNA before (lane 1) and after (lane 2–6) cleavage with a specific restriction endonuclease. This enzyme cleaves the normal control DNA (lanes 2 and 3) at a single site; however, A985→G (K304E), the common *MCAD* mutation, creates an additional recognition site for the enzyme. Thus, individuals who are heterozygotes (lanes 4 and 5) and homozygous (lane 6) for the mutation can be easily identified.

et al. 1992; Gray and Green 1994; Stone and Sidransky 1999; Preece and Green 2002). The IEMs that may present as hydrops in a fetus or infant and their modes of diagnosis are summarized in Table 7.1. Most of these disorders involve lysosomal storage disorders or red blood cell enzymatic deficiencies causing hemolytic anemias. An interesting exception to this is Pearson's marrow-pancreas syndrome (Fig. 7.6). This condition is caused by a deletion of mitochondrial DNA causing a generalized disorder of oxidative-phosphorylation with particular effect on the bone marrow. The main clue to the presence of a metabolic cause for NIH comes from histological examination of fetal tissues. In Pearson syndrome ring sideroblasts are present in marrow preparation (Fig. 7.6). In lysosomal storage disorders placental villi often show storage vacuolation (Fig. 7.7). A recent bulletin from the British Inherited Metabolic Disease Group (BIMDG) gives a useful summary of the recommended sampling and testing in this group (http://www.bimdg.org.uk/bulletins/summer2004/BIMDG26_Neonatal_Hydrops_GG.pdf).

Maternal Intoxication

It has been known for many years that placental transport of metabolites that have accumulated as a result of maternal IEM may produce an embryopathy in a fetus. The best known examples of this is the deleterious pregnancy outcomes associated with poorly controlled maternal PKU. About 80% of exposed fetuses have microcephaly, mental

TABLE 7.1. Inborn errors of metabolism presenting as hydrops fetalis

Disorder	Enzyme deficiency	Diagnostic assay	Comment
Lysosomal disorders			
MPS I (Hurler)	α-L-iduronidase	CVS, Fib, WC, Amn	Chr 4p16.3
MPS IV (Morquio A)	Galactose-6-sulfatase	CVS, Fib, WC	Chr 16q24
MPS VII (Sly)	β-glucuronidase	Fib, Amn	Chr 7q21
Mucolipidosis II (I-cell)	*N*-acetylglucosamine-1-phosphotransferase	Fib, Amn	
Sialidosis II	Neuraminidase	Fib, Amn	Chr 10pter-q23
Galactosialidosis	Cathepsin A,β-galactosidase + neuraminidase	Fib, Amn	Chr 20q13
Niemann-Pick type C	Intracellular cholesterol trafficking	Fib	Chr18q12 and Chr14q24
Gaucher type II	Acid β-glucosidase	CV, Fib, WC, Amn	Chr 1q21
GM_1 gangliosidosis	β-galactosidase	CV, Fib	Chr 3p
Mitochondrial disorders			
Pearson's	General mitochondrial dysfunction	mtDNA deletions in all tissues	
Glucose metabolism			
G-6-PD	Glucose-6-phosphate dehydrogenase	RBC	Chr Xq28 single case
Pyruvate kinase	Pyruvate kinase	RBC	Chr 1q21 and 15q22
GPI	Glucose phosphate isomerase	RBC	Chr 19q

Abbreviations in diagnostic assay column refer to the tissues in which the enzymatic activity may be assayed: CV, chorionic villus; Fib, fibroblasts; WC, white cells; Amn, cultured amniocytes; RBC, red blood cells.

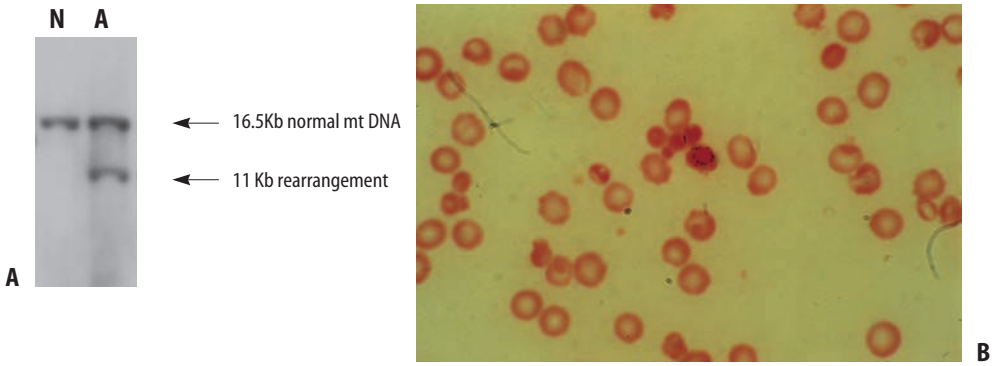

Figure 7.6. Pearson's marrow-pancreas syndrome. (A) Southern blot analysis showing normal (lane N) and affected (lane A) individual with a 5.5-kilobase (kb) deletion of mitochondrial DNA. (B) Ring sideroblasts found in the marrow sample of a hydropic neonate with Pearson's syndrome.

retardation, and impaired somatic growth. Malformations of the heart and other structures are seen in about 20% (Cipcic-Schmidt et al. 1996; Levy 1996).

Recently, it has been recognized that metabolic disease in the fetus can cause serious maternal disease. The HELLP syndrome (hypertension, elevated liver enzymes, and low platelets) is a potentially life-threatening disorder that can occur in mothers carrying a fetus with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (Sims et al. 1995); LCHAD is one of the enzymes involved in mitochondrial β-oxidation of fatty acids. In postnatal life it results in episodic nonketotic hypoglycemia, cardiomyopathy, and hepatic dysfunction. Interestingly, a common LCHAD mutation (E474Q) is always present on one allele of the affected fetus where the mother has HELLP, but the second mutation is variable. Why the acute maternal illness occurs and, in particular, why it occurs with this specific mutation, is not clear.

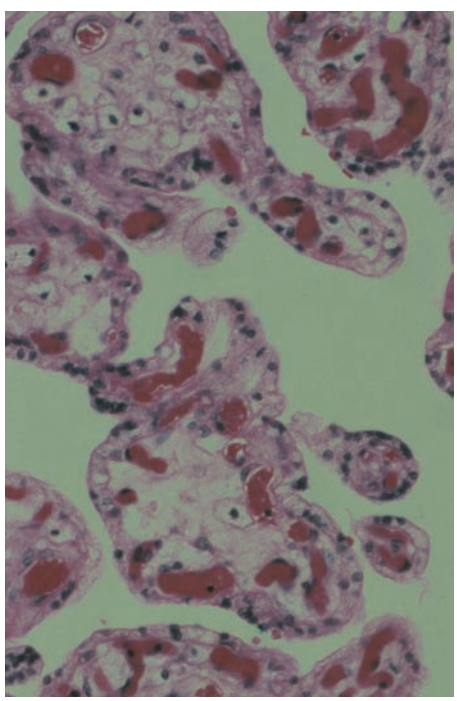

Figure 7.7. A section through placental villi from a hydropic infant affected with GM_1 gangliosidosis, a lysosomal storage disorder. There is cytoplasmic vacuolation.

Clinical Presentations of Inborn Errors of Metabolism in the Neonatal Period

When aiming for early diagnosis of neonatal IEMs, as in all mendelian disease in childhood, it is important to bear the following points in mind:

- Each condition is individually rare but collectively common.
- Aspects of the family history may be particularly helpful (e.g., parental consanguinity, neonatal death in siblings, or maternally related male relatives).
- Inborn errors of metabolism should not be considered diagnoses of last resort, and a structured approach to the diagnosis should be sought for particular presentations.

One of the challenges of neonatology is the limited repertoire of clinical responses to illness seen in the newborn. Selected IEMs may present as a positive result from the national neonatal screening program. However, in neonates with IEMs the mode of acute presentation most often falls into one of the following categories:

- The intoxicated infant: appears normal in the perinatal period followed by a period of rapid generalized deterioration usually after feeding is established.
- Prominent visceral involvement: most often involving isolated hepatic or cardiac dysfunction.
- The congenitally "floppy baby" (with or without seizures): tend to be recognized as abnormal from birth.
- Multiple malformation syndromes.

The Intoxicated Baby

In this group a characteristic history would be of a full-term infant with a normal birth weight who is well for the first 2 or 3 days of life and then refuses feeds, has recurrent vomiting, and becomes hypertonic or comatose. Unless specific treatments are instituted, such a clinical picture would usually progress rapidly to death. A favorite fact cherished by medical students is that in some of these disorders the abnormal metabolites may be recognized by an unusual odor (Table 7.2). A more useful diagnostic approach can be made on the basis of acid-base balance, glucose/lactate homeostasis, and ammonia level (Fig. 7.8). A list of IEMs known to present in fetal and neonatal life is given in Table 7.3. Saudubray et al. (1989, 2002) reported a 20-year survey of IEMs presenting in the neonatal period and showed that despite the large number of possible diagnoses, 72.5% of the cases were due to seven disorders in four categories:

- Aminoacidopathies (maple syrup urine disease and nonketotic hyperglycinemia), usually diagnosed by plasma amino acid chromatography.
- Organic acidurias (methylmalonic aciduria, isovaleric aciduria and propionic aciduria), diagnosed by urinary organic analysis.
- Hyperammonemias (ornithine transcarbamylase deficiency and citrullinemia), usually diagnosed by plasma ammonia estimation and amino acid chromatography.
- Congenital hyperlactacidemias (disorders of pyruvate metabolism, respiratory chain disorders), usually diagnosed by serum lactate and pyruvate measurement and specific enzymes assays.

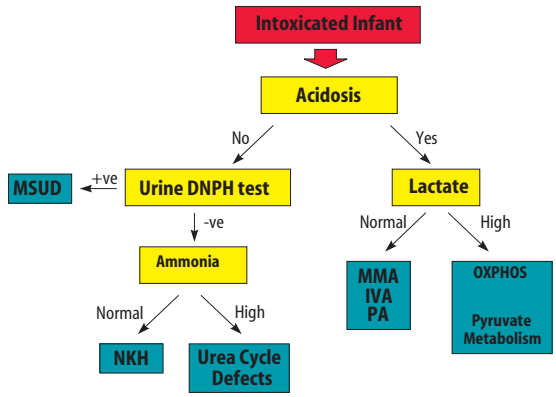

FIGURE 7.8. Simplified diagnostic algorithm for investigation of the neonate presenting with intoxication.

TABLE 7.2. Inborn errors of metabolism as a case of unusual odors

Disorder	Odor
Phenylketonuria	Musty or mousy
Hereditary tyrosinemia	Musty or cabbage-like
Maple syrup urine disease	Maple syrup or burnt sugar
Isovaleric acidemia	Cheesy or sweaty feet
Glutaric aciduria type II	Sweaty feet
Multiple carboxylase deficiency	Cat urine

Aminoacidopathies

Maple Syrup Urine Disease

Maple syrup urine disease (MSUD) is the commonest single IEM presenting in the neonatal period, accounting for 12% of all cases. It is caused by homozygosity for mutations in the genes encoding the subunits of the branched chain α-keto acid dehydrogenase complex (this has a similar structure to that of pyruvate dehydrogenase; see The Intoxicated Baby, above). These mutations (which may affect any of the subunits) result in accumulation of the branched chain

amino acids (leucine, isoleucine, and valine). Unusual clinical features include boxing and pedaling movements and the lack of significant acidosis. The diagnosis of MSUD is based on the greatly increased levels of these amino acids in the blood, CSF, and urine, particularly leucine.

Nonketotic Hyperglycinemia

In nonketotic hyperglycinemia (NKH) abnormal quantities of glycine accumulate in all body tissues as a result of an inborn error of the glycine cleavage pathway (Fig. 7.2C). Mutations have been identified in the components (P, H, T, and L proteins) of a mitochondrial enzyme complex. Most patients presenting in the neonatal period have a defect in the P protein (Applegarth and Toone 2001). Seizures are a prominent component of this disorder and are often associated with a characteristic burst-suppression pattern on the electroencephalogram (EEG) (Hoover-Fong et al. 2004). The diagnosis is usually established by demonstrating a CSF/plasma glycine ratio of greater than 0.08. Organic acid analysis should be performed to exclude propionic acidemia (ketotic hyperglycinemia). Enzymatic confirmation requires the measurement of glycine cleavage activity in the liver. Nonketotic hyperglycinemia accounts for about 10% of IEMs presenting in the neonatal period.

Organic Acidurias

Methylmalonic Aciduria

Methylmalonic aciduria (MMA) is a product of dysfunction of the enzyme methylmalonyl CoA mutase. This catalyses the conversion of methylmalonyl CoA to succinyl CoA and is an adenosylcobalamin (a vitamin B_{12} derivative) dependent enzyme. Thus mutations in the genes encoding either the mutase enzyme itself or the enzymes involved in cobalamin synthesis can produce MMA. The diagnosis is based on the presence of urinary methylmalonate and specific enzyme assays (Ogier de Baulny and Saudubray 2002; Horster and Hoffmann 2004).

Isovaleric Aciduria

Isovaleric aciduria (IVA) is due to isovaleryl-CoA dehydrogenase deficiency. This enzyme converts isovaleryl-CoA to 3-methylcrotonyl-CoA as a intermediary step in the catabolism of leucine. The diagnosis is made by demonstrating isovalerylglycine in urine. It is this substance that gives these patients the characteristic "sweaty feet" smell (Ogier de Baulny and Saudubray 2002).

Propionic Aciduria

Propionyl CoA carboxylase is a biotin-dependent enzyme that catalyzes the conversion of propionyl CoA (a by-product of the catabolism of several amino acids, odd-chain fatty acids, and cholesterol) to methylmalonyl CoA. Deficiency of this enzyme results in massive accumulation of propionic acid in all tissues, and such patients often show a ketotic hyperglycinemia that is currently unexplained. Diagnosis is made on the finding of urinary propionate and demonstration of enzyme deficiency (possible in many tissues including liver, leucocytes and fibroblasts (Ogier de Baulny and Saudubray 2002).

Hyperammonemias

In this category the most common IEMs presenting in the neonatal period are defects in the urea cycle, which account for 21% of IEMs presenting in the neonatal period. This enzymatic cycle is present in the liver and intestine and converts toxic nitrogenous waste into the water-soluble compound urea. A severe deficiency in this cycle results in a typical history of a full-term baby who becomes acutely unwell (lethargic, hypothermic tachypneic, and refusing feeds) on day 4 with a respiratory alkalosis and very high plasma ammonia. Such a presentation may be rapidly fatal even with full supportive therapy. Ornithine transcarbamylase (OTC) deficiency is caused by mutations in the OTC gene located on Xp21. Ornithine transcarbamylase catalyzes the formation of citrulline from ornithine and carbamyl phosphate. Thus deficiency results in hypocitrullinemia and high levels of urinary orotic acid, a product of carbamyl phosphate metabolism by the pyrimidine synthetic pathway. These biochemical features are very useful in making the diagnosis, which may be confirmed by enzyme assay in the liver. Citrullinemia is caused by deficiency of argininosuccinate synthetase (ASS). The ASS gene is located on 9q34. The diagnosis is usually made on plasma amino acid analysis by finding plasma

Table 7.3. Inborn errors of metabolism presenting in the neonatal period

Disorder	Enzyme deficiency	Hydrops	Intoxication	Visceral	Malformations
Lysosomal disorders					
MPS I (Hurler)	α-L-iduronidase	✓		✓	
MPS IV (Morquio A)	Galactose-6-sulfatase	✓		✓	
MPS VII (Sly)	β-Glucuronidase	✓		✓	
Mucolipidosis II (I-cell)	*N*-acetylglucosamine-1-phosphotransferase	✓		✓	
Sialidosis II	Neuraminidase	✓		✓	
Galactosialidosis	Cathepsin A, β-galactosidase + neuraminidase	✓		✓	
Niemann-Pick types A & B	Sphingomyelinase			✓	
Niemann-Pick type C	Intracellular cholesterol trafficking	✓		✓	
Gaucher type II	Acid β-glucosidase	✓		✓	
GM_1 gangliosidosis	β-Galactosidase	✓		✓	
Wolman disease	Acid lipase			✓	
Farber disease	Ceramidase			✓	
Fucidosis	α-Fucosidase			✓	
Mitochondrial disorders					
Pearson's	General mitochondrial dysfunction	✓	✓		
Carbohydrate metabolism					
G-6-PD	Glucose-6-phosphate dehydrogenase	✓			
Pyruvate kinase	Pyruvate kinase	✓			
GPI	Glucose phosphate isomerase	✓			
Galactosemia	Galactose-1-phosphate uridyl transferase		✓	✓	
Fructose intolerance	Fructose-1-phosphate aldolase		✓		
GSD I	Glucose-6-phosphatase			✓	
GSD II	α-1,4-glucosidase			✓	
GSD III	Debrancher enzyme			✓	
GSD VI	Brancher enzyme			✓	
PDH	Pyruvate dehydrogenase		✓		✓
Pyruvate carboxylase	Pyruvate carboxylase		✓		
PEPCK	Phosphoenolpyruvate carboxykinase		✓		
Urea cycle defects					
CPS	Carbamyl phosphate synthetase		✓		
OTC	Ornithine transcarbamylase		✓		
Citrullinemia	Arginosuccinic acid synthase		✓		
Arginosuccinic aciduria	Arginosuccinic acid lyase		✓		
Arginase	Arginase		✓		
Amino acid metabolism					
Maple syrup urine disease	α-Keto acid dehydrogenase complex		✓		✓
Hypervalinemia	Valine transamination		✓		
Periodic hyperlysinemia	α-Aminoadipic semialdehyde synthase		✓		
Hyper-b-alaninemia	4-Aminobutyrate α-ketoglutarate aminotransferase				
NKH	Glycine cleavage complex		✓		✓
Tyrosinemia	Fumarylacetoacetate hydrolase		✓		
Pyroglutamic aciduria	Generalized glutathione synthetase deficiency		✓		
Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH)	?		✓		
Lysinuric protein intolerance	?		✓		
MTHFR	Methylenetetrahydrofolate reductase		✓		✓
Sulfite oxidase	Sulfite oxidase		✓		
Organic acid metabolism					
Methylmalonic aciduria	Methylmalonyl CoA mutase + others		✓		
Propionic acidemia	Propionyl CoA carboxylase		✓		✓
Isovaleric acidemia	Isovaleryl-CoA dehydrogenase		✓		
3-Methyl crotonyl CoA carboxylase	3-Methyl crotonyl CoA carboxylase		✓		

Table 7.3. *Continued*

Disorder	Enzyme deficiency	Hydrops	Intoxication	Visceral	Malformations
Multiple carboxylase	Holocarboxylase synthetase		✓		
Glutaric aciduria type II	Multiple acyl-CoA dehydrogenase		✓		✓
Ethylmalonic-adipic aciduria	?		✓		
HMG aciduria	Hydroxymethylglutaryl CoA lyase		✓		
2-Methyl-3-hydroxybutyric aciduria	β-Ketothiolase		✓		
D-glyceric acidemia	? D-glycerate kinase		✓		
Peroxisomal disorders					
Zellweger	Peroxisomal assembly				✓
Pseudo-Zellweger	VLCFA β-oxidation				✓
RCDP	Peroxisomal protein import				✓
Fatty acid metabolism					
MCAD	Medium-chain acyl-CoA dehydrogenase		✓		
Systemic carnitine deficiency	? Carnitine transporter		✓		
Other disorders					
Menkes	ATP7A				✓
Neonatal hemochromatosis	?			✓	
α_1-AT	α_1-Antitrypsin			✓	

citrulline levels between 1000 and 5000 µmol/L (normal, 10 to 20 µmol/L). Organic acid analysis should always be performed, as a number of organic acidemias may also present with hyperammonemia, notably propionic and methylmalonic acidurias.

Congenital Hyperlactatemias

Lactic acid is the product of anaerobic metabolism of glucose via enzymatic reduction of pyruvate (by lactate dehydrogenase) and can only be removed by a reversal of this process. The normal lactate/pyruvate ratio in human plasma is between 10:1 and 25:1. High lactate levels can be caused by many nongenetic diseases, including tissue necrosis and sepsis. This is due to the reduced level of oxygen in tissues that normally rely on oxidative metabolism of glucose. Hypoxia produces this effect by inhibiting the production of adenosine triphosphate (ATP) by oxidative phosphorylation while at the same time stimulating the production of lactate by increasing the rate of glycogenolysis. This produces a much increased lactate/pyruvate ratio.

Two groups of IEMs commonly present with congenital hyperlactatemia: disorders of pyruvate metabolism and oxidative phosphorylation (OxPhos) disorders. Combined, these account for 13% of IEMs presenting in the neonatal period. Other IEMs can present with high lactate levels; however, this is a secondary phenomenon and can be easily diagnosed by the presence of abnormal organic acids.

Disorders of Pyruvate Metabolism

There are two important deficiencies in this group: pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC). Pyruvate dehydrogenase is a multienzyme complex made up of four subunits (E1α, E1β, E2, and E3) and catalyzes the conversion of pyruvate to acetyl-CoA. Most PDH deficiencies are due to mutations in the *E1α* gene on Xp22.1-22.2, although mutations in the three other subunits have been described. Pyruvate carboxylase catalyzes the conversion of pyruvate to oxaloacetic acid (OAA). Hemizygous males with PDH or homozygous deficiency of PC present in the newborn period with a rapidly fatal hyperlactatemia. It should be noted that females heterozygous for PDH deficiency may present with a dysmorphic malformation syndrome (see above). These disorders then produce high lactic acid levels with a normal lactate/pyruvate ratio (i.e., concordantly high pyruvate levels). Both PC and PDH can be assayed successfully in cultures fibroblasts.

Oxidative Phosphorylation Disorders

Oxidative phosphorylation is a remarkable intramitochondrial process responsible for producing the majority of the ATP required for normal

cellular function. Five enzyme complexes (complexes I to V) mediate OxPhos activity. These complexes are made up of many different polypeptides; 13 of these are encoded in the mitochondrial genome and about 70 in the nuclear genome. Defects in any of these components may produce an OxPhos disease. As would be predicted by the complexity of the system, OxPhos disorders have a bewildering array of clinical presentations (Smeitink 2003; Zeviani and Spinazzola 2003; Dimauro and Gurgel-Giannetti 2005). These disorders should be suspected in any infant with hyperlactatemia with normal organic acids and a high lactate/pyruvate ratio in blood or CSF.

Prominent Visceral Involvement

Isolated visceral involvement as a neonatal presentation of IEM is relatively rare. The two commonest organs involved are the liver (Clayton 2002) and the heart. Cholestatic jaundice can be the presenting symptom in α_1-antitrypsin deficiency and inborn errors of bile acid metabolism. Hepatomegaly with generalized liver failure suggests tyrosinemia type I, galactosemia, neonatal hemochromatosis, or respiratory chain disorders (see The Intoxicated Baby, above). Hepatomegaly with hypoglycemia is the usual presentation of glycogen storage disorders. Hepatosplenomegaly in the newborn period is rare but may be seen in GM_1 gangliosidosis, Gaucher's disease, Niemann-Pick disease, and Wolman disease. Cardiomyopathy can be a presenting problem in respiratory chain disorders, fatty acid oxidation disorders, congenital disorders of glycosylation, and glycogen storage diseases (Schwartz et al. 1996; Schmaltz 2001; Roe et al. 2002; Gehrmann et al. 2003).

The Floppy Baby (with or Without Seizures)

Profound central hypotonia is an unusual but highly characteristic presentation of IEM. This clinical picture is usually present from birth and is often associated with seizures and other neurological abnormalities and usually rapidly causes death. This presentation probably reflects prenatal cerebral damage or malformation. The common diagnoses in this group are the following:

- Disorders of peroxisomal function (see The Baby with Malformations, below)
- Menkes' disease

Congenital hyperlacticacidemia may also present in this way although they more commonly present as intoxications (see The Intoxicated Baby, above). The non-IEM differential diagnoses include sepsis, birth asphyxia, CNS malformations (see The Baby with Malformations, below), Prader-Willi syndrome, chromosome abnormalities, and transplacental exposure to medications.

Menkes' Disease

Menkes' disease is a rare X-linked disorder of copper transport. The clue to this diagnosis is in the steely appearance of the hair (pili torti) in affected individuals. The diagnosis is made by demonstration of low levels of serum copper and ceruloplasmin. The causative gene has been cloned, and a predicted protein (termed ATP7A) has features characteristic of the family of cation translocating membrane proteins termed P-type adenosine triphosphatases (ATPases) (Kaler 1998a,b; Gasch et al. 2002).

The Baby with Malformations

Major malformations are present in 2.5% of births. The cause of the majority of these birth defects is not known. There has been an recent appreciation that a small but significant group of IEMs can present as isolated CNS malformations or multiple malformation syndromes (Brown 1994; Schwartz et al. 1996; Nissenkorn et al. 2001). The reason these rare disorders are of importance is because they provide a link between inborn errors of metabolism and inborn errors of morphogenesis. It would appear that the developing brain and in particular neuronal migration is a particular target for IEM embryopathy. An understanding of the pathophysiology of these disorders is likely to shed light on the developmental processes in the normal brain.

Central Nervous System Malformations

The IEMs that cause isolated CNS malformations are summarized in Table 7.4. Pachy- or microgyria appears to be the commonest malformation

Table 7.4. Inborn errors of metabolism causing CNS malformations

Disease	Agenesis of corpus callosum	Hydrocephalus	Micro- or pachygyria	Cerebellar hypoplasia
3–hydroxyisobutyric aciduria			✓	
Carbohydrate deficient glycoprotein syndromes		✓		✓
Fumarase deficiency	✓	✓		
Glutamate forminotransferase		✓		
Glutaric aciduria II			✓	
HHH syndrome				✓
Maple syrup urine disease			✓	
Methylenetetrahydrofolate reductase deficiency			✓	
Molybdenum cofactor deficiency			✓	
Nonketotic hyperglycinemia	✓		✓	
Pyruvate dehydrogenase deficiency	✓			
Respiratory chain disorders	✓		✓	
Zellweger syndrome and associated phenotypes			✓	

followed by agenesis of the corpus callosum, hydrocephalus, and cerebellar hypoplasia. It may be difficult to differentiate a dysplastic cause of abnormal CNS morphology from a true malformation. This problem is best illustrated by the hydrocephalus that develops in the early postnatal period in several lysosomal storage diseases. If the dysplastic condition has a prenatal onset, then this makes correct categorization even more problematic. For this reason the disorders associated with hydrocephalus and cerebellar hypoplasia should be considered as possible dysplasias.

Pyruvate dehydrogenase heterozygosity is the best-studied IEM associated with CNS malformations with partial or total agenesis of the corpus callosum being the predominant feature (Fig. 7.9). Facial dysmorphisms are also present in about one third of these cases of PDH complex deficiency and are thought to resemble those of fetal alcohol syndrome. The cause of these malformations is not clear but the process of X-inactivation would lead to a population (about 50%) of cells in the developing brain of a PDH heterozygote that had severe PDH deficiency. These cells may be at a particular growth disadvantage during neurogenesis, which may result in structural abnormalities.

Central Nervous System and Other Malformations

3-Hydroxyisobutyryl-CoA Deacylase Deficiency

A single case has been described of a male infant with dysmorphic features, poor feeding, failure to thrive, hypotonia, and severe neurodevelopmental delay. Other malformations included vertebral abnormalities, tetralogy of Fallot, and agenesis of the corpus callosum. This is a disorder of valine metabolism. The diagnosis is made by detecting unusual sulfur amino acids [S-(2-carboxypropyl)-cysteine and S-(2-carboxypropyl)-cysteamine] in the urine. 3-Hydroxyisobutyryl-CoA deacylase was found to be deficient in this case when measured in liver and fibroblasts. This is presumably an autosomal recessive disorder because both parents had activities of 3-hydroxyisobutyryl-CoA deacylase of about 50% of normal, as would be expected for heterozygotes.

Cholesterol Synthesis In Development

An unexpected role for endogenous cholesterol biosynthesis during human development has become apparent with the identification of the following autosomal and X-linked recessive malformation syndromes associated with disorders of this pathway (Porter 2003):

- Mevalonate kinase deficiency (malonic aciduria and Hyper-IgD syndrome [HIDS])
- 7-Dehydrocholesterol reductase deficiency (Smith-Lemli-Opitz syndrome)
- 24-Sterol reductase deficiency (desmosterolosis)
- 3-Sterol dehydrogenase (C4 decarboxylase) deficiency (CHILD syndrome: congenital hemidysplasia with ichthyosiform erythroderma and limb defects)

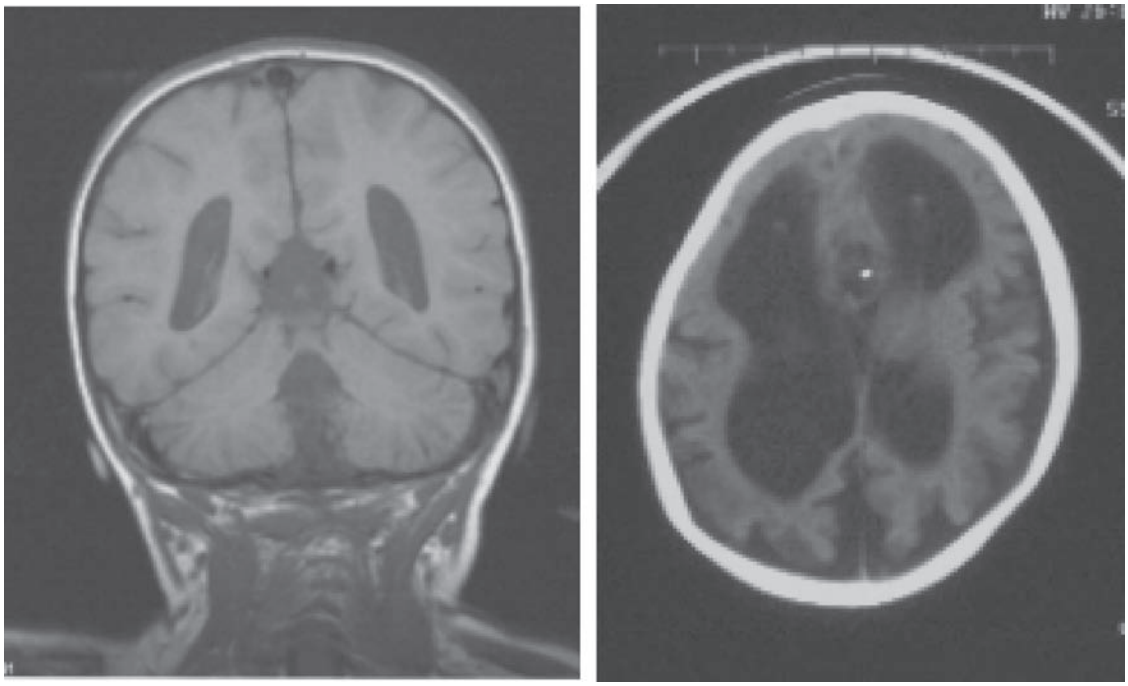

Figure 7.9. Central nervous system malformations. Computed tomography (CT) scans of brain in females heterozygous for mutation in the pyruvate dehydrogenase gene showing agenesis of the corpus callosum (A) and ventriculomegaly (B).

- 8(7)-Sterol isomerase deficiency (Conradi-Hunermann syndrome and CHILD syndrome)
- C5-Desaturase deficiency (lathosterolosis)

There are two general mechanisms by which aberrant cholesterol synthesis may cause developmental pathology: a relative deficiency of cholesterol and a relative excess of the sterol precursor. These are not mutually exclusive. Abnormal sterols are known to alter membrane fluidity, which may alter both the movement of embryonic cells and cell-cell interaction. Another possibility is that altering the sterol content of membranes may lead to the aberrant functioning or mis-targeting of some proteins. Recently the posttranslational attachment of cholesterol to mammalian hedgehog proteins has been demonstrated. These are an important family of proteins with potent morphogenic activity. The interruption of posttranslational modification suggests a possible mechanism for the embryopathy seen in these biochemical disorders. For the sake of brevity, only the first three disorders are mentioned in detail here.

Mevalonate Kinase Deficiency

Mevalonic aciduria was first described in the mid-1980s by two groups. One case with cerebellar ataxia was in a 2-year-old boy with severe failure to thrive, developmental delay, anemia, hepatosplenomegaly, central cataracts, and dysmorphic facies. Mevalonate kinase deficiency was subsequently confirmed in this family. This is an apparently rare and often lethal disorder with a relapsing and progressive case. One of the most striking pathological features is cerebellar atrophy. The failure to thrive and anemia that are seen may be a result of the failure of the cell to provide substrate for posttranslational modification (isoprenylation) of important growth promoting proteins such as $p21^{ras}$.

Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz (SLO) syndrome is an autosomal recessive condition that in its most severe form is a lethal multiple malformation syndrome characterized by microcephaly, cleft

palate, cardiac malformations, polydactyly, genitourinary anomalies, and 2/3 syndactyly (Fig. 7.10). The accumulation of 7-dehydrocholesterol (7DHC), an immediate biosynthetic precursor of cholesterol, is a biochemical marker for this syndrome due to 7DHC reductase deficiency.

Desmosterolosis

There are only two known cases of this condition, which is due to an autosomal recessive deficiency of 3-beta-hydroxysterol delta-24-reductase (Waterham et al. 2001; Andersson et al. 2002). The

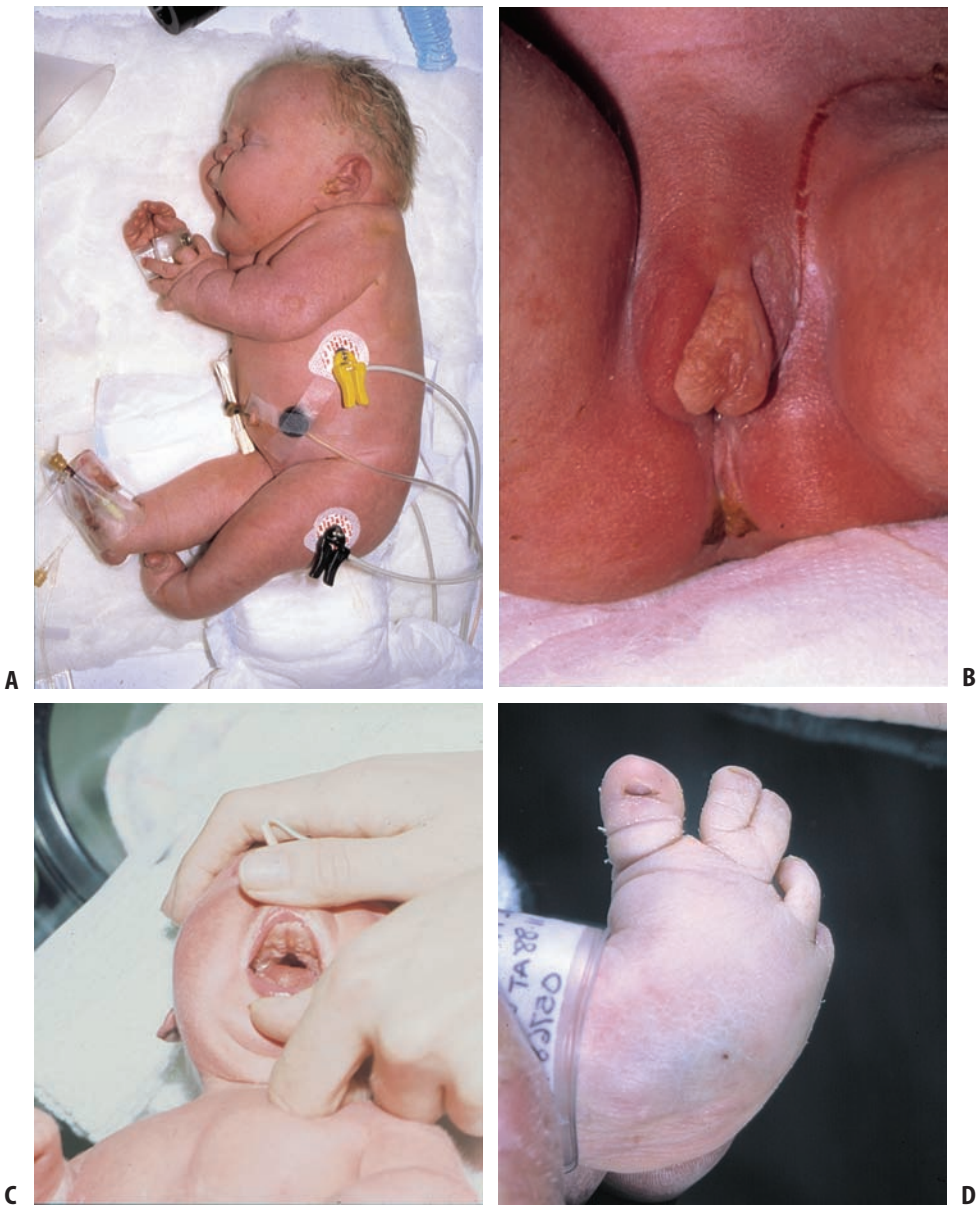

Figure 7.10. Smith-Lemli-Opitz (SLO) syndrome. (A) Phenotype of severe SLO in a neonate with micrognathia, short neck, polydactyly, and arthrogryposis. (B) Ambiguous genitalia in an infant with a 46XY karyotype. (C) Marked palatal rugae seen in SLO. (D) 2/3 syndactyly in feet of affected individual.

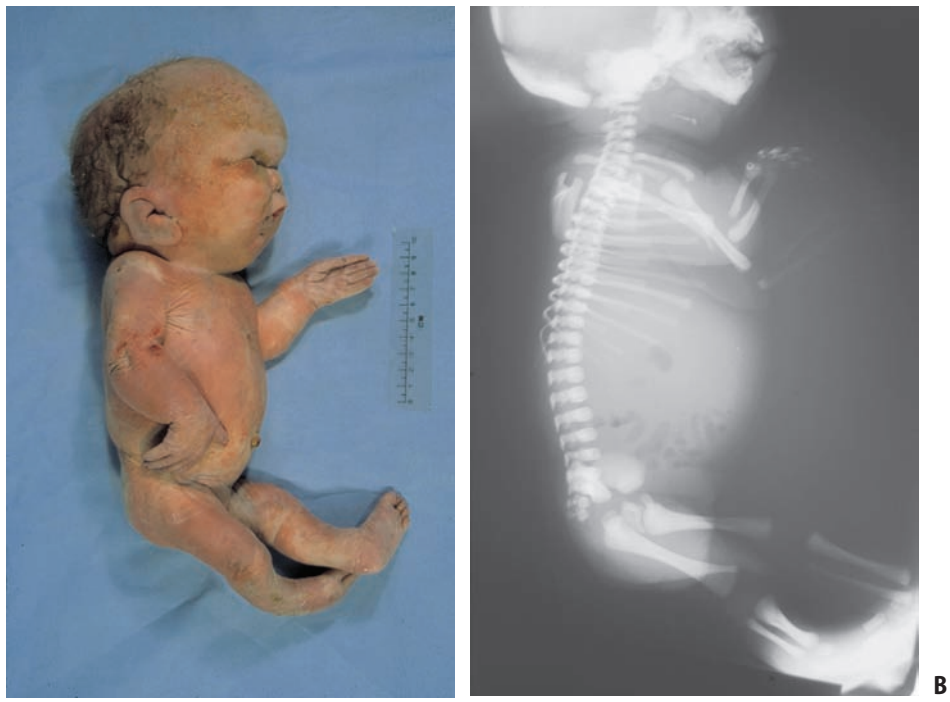

Figure 7.11. Clinical phenotype of desmosterolosis. (A) Lateral photograph showing macrocephaly, low-set ears, and short limbs. (B) X-rays of the same child showing generalized osteosclerosis.

first patient, who died within 1 hour of birth, was macrocephalic with marked frontal bossing and low-set ears. She had a cleft palate, gingival nodules, total anomalous pulmonary venous drainage, bilateral renal hypoplasia, and an unrotated small bowel. The brain showed an immature gyral pattern with poor development of the corpus callosum and gross dilation of the ventricular system. X-rays revealed generalized osteosclerosis with rhisomesomelic shortening of all four limbs (Fig. 7.11). GC-MS analysis of all tissues showed two major peaks (Fig. 7.12). The first was cholesterol but the second (and larger) peak had a retention index and mass spectrum identical with that of desmosterol. The second case had severe microcephaly, agenesis of the corpus callosum, downslanting palpebral fissures, micrognathia, submucous cleft palate, clubfoot, and a persistent patent ductus arteriosus (Andersson et al. 2002).

Disorders of Peroxisomal Structure and Function

Peroxisomes are intracellular membrane-bound metabolic compartments found throughout the eukaryotic kingdom. Peroxisomes are roughly spherical organelles bound by a single lipid bilayer with a diameter of 0.1 to 1 μm. The enzymatic abilities of human peroxisomes can be divided into five broad and overlapping categories:

- Simple oxidases (e.g., D-amino acid oxidase, polyamine oxidase) producing heat and H_2O_2, which is decomposed by catalase
- β-oxidation cycles for degradation of very long chain fatty acids (VLCFAs), pristanic acid, and bile acid intermediates
- The glyoxalate cycle, which catalyzes the conversion of acetyl-CoA to succinate (this is of uncertain significance in humans)
- Ether lipid synthesis pathway
- Cholesterol and dolichol biosynthesis

Zellweger (Cerebrohepatorenal) Syndrome

Zellweger syndrome (ZS) was originally described as a lethal, multiple malformation syndrome of infancy. Genetic mutations causing either a generalized disorder of peroxisomal function

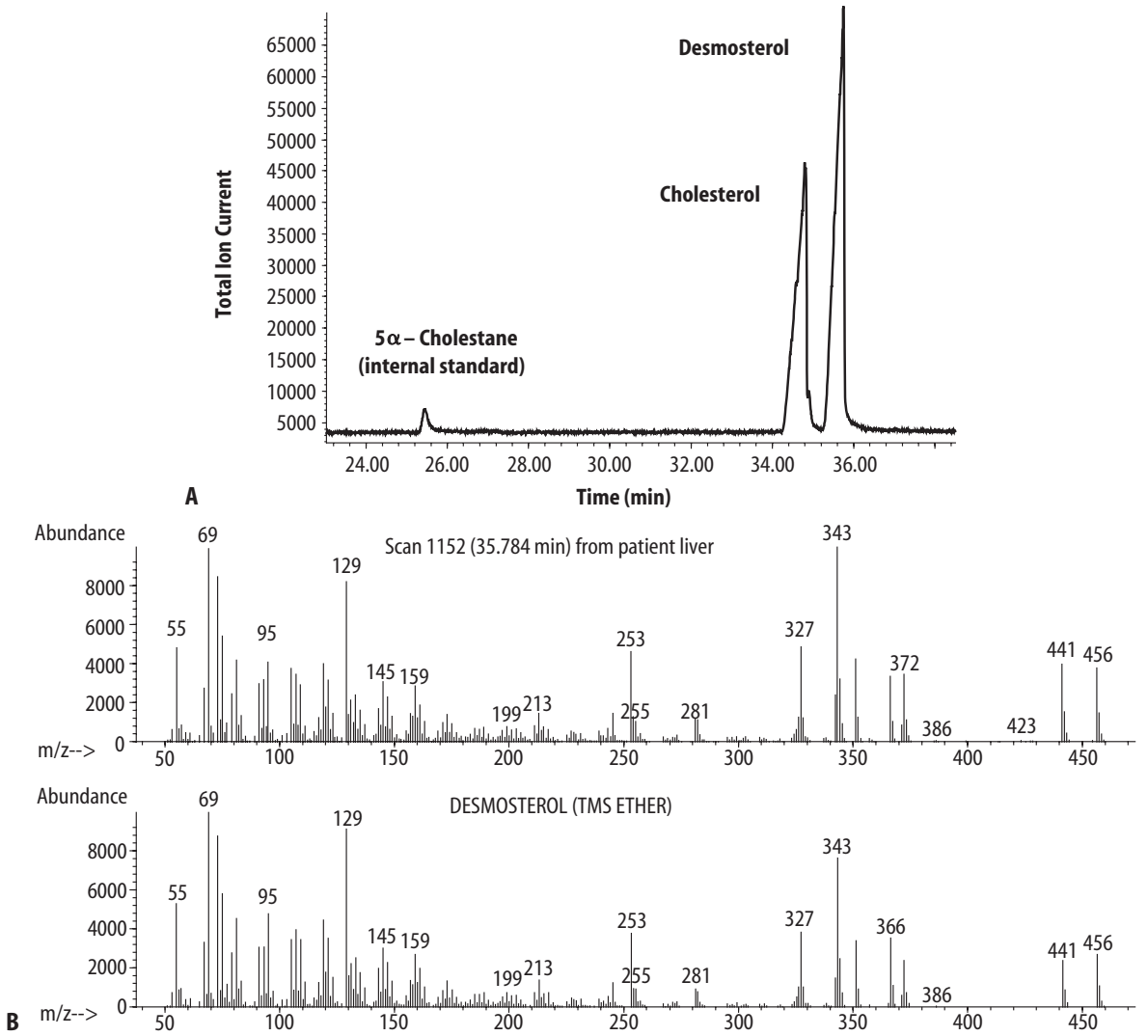

FIGURE 7.12. Diagnosis of cholesterol biosynthesis disorders using gas chromatography and mass spectrometry (GC-MS). Normal tissues show one major peak, cholesterol. (A) In desmosterolosis tissues show two major peaks, the first being cholesterol and the second (and larger) peak desmosterol. (B) The mass spectrum of this second peak is identical to that of chemically pure desmosterol.

(i.e., interrupting peroxisome assembly) or single matrix enzyme deficiencies can cause a similar spectrum of abnormalities (Wanders and Waterham 2005). Many cases of ZS present with profound congenital hypotonia and no psychomotor development whatsoever. The basis of this severe cerebral dysfunction appears to be the premature arrest of migrating neuroblasts during development, resulting in site-specific cerebral micro- and pachygyria with neuronal heterotopia. The cortical regions showing the most severe abnormalities are the perisylvian and frontoparietal areas (Fig. 7.13).

The other clinical features of ZS have been well defined (FitzPatrick 1996). Hepatomegaly is seen in about 80% of infants with ZS. Liver biopsy may reveal a micronodular cirrhosis and giant cell formation with or without hepatic fibrosis (Fig. 7.13). Prenatal onset renal cortical cysts of variable size are seen in about 70% of cases (Fig. 7.13). About

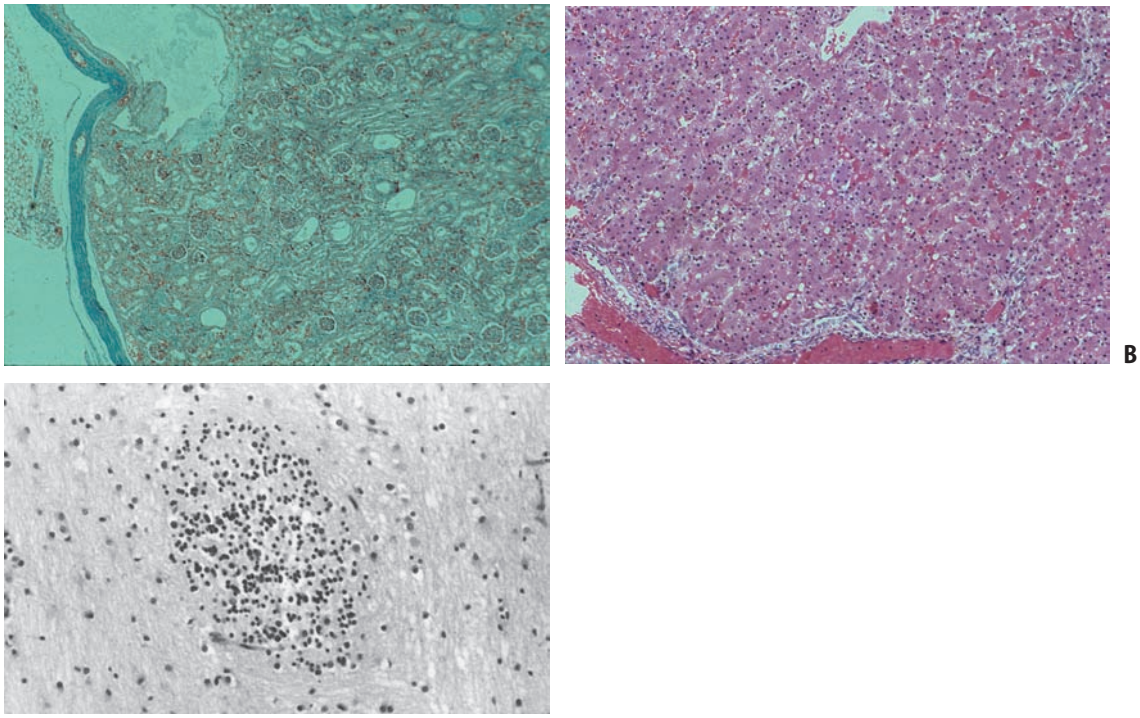

Figure 7.13. Zellweger syndrome (ZS). (A) Hematoxylin and eosin (H&E) stain showing renal cortical cysts. (B) H&E stain of liver tissue showing micronodular cirrhosis. (C) H&E stain showing abnormal foci of migrating neuroblasts in the brain of an infant with ZS.

90% of ZS cases have congenital sensorineural hearing impairment. Ocular findings include abnormal electroretinogram (ERG) (85%), cataracts (70%), peripheral pigmentary retinopathy (40%), and optic nerve hypoplasia (40%). The main radiological finding in ZS is calcific stippling of the patellae (Fig. 7.14) with synchondrosis of the acetabulum. Neonatal adrenoleukodystrophy (NALD), infantile Refsum's disease (IRD), and hyperpipecolic acidemia (HPA) are the names given to similar disorders that tend to have less severe clinical phenotypes than ZS but with similar organ involvement, biochemical phenotype and pathogenesis.

Pseudo–Zellweger syndrome (PZS) was originally used to describe a unique case with clinical features of ZS, structurally normal peroxisomes (Goldfischer et al. 1986). Mutations in each of the enzymes involved in VLCFA β-oxidation (peroxisomal 3-oxoacyl CoA thiolase, acyl-CoA oxidase, and trifunctional enzyme) may cause PZS. The combined birth prevalence of ZS and PZS is

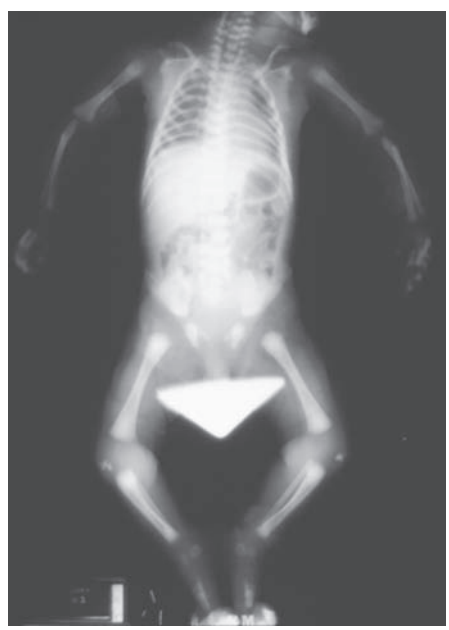

Figure 7.14. Radiology of peroxisomal dysfunction. X-rays of a neonate with Zellweger syndrome showing patellar stippling.

Table 7.5. Postmortem investigations for inborn errors of metabolism

Sample	Procedure
Urine	Store sample at −20°C for future analysis
Blood	2- to 5-mL samples of serum and plasma; freeze aliquots at −20°C
	5- to 10-mL ethylenediaminetetraacetic acid (EDTA) sample for DNA extraction; freeze whole blood at −20°C
CSF	1-mL sample, freeze at −20°C
Liver and muscle	Snap freeze at −70°C for enzyme analysis
Cell culture	Establish fibroblast culture from skin, pericardium, or other tissue

thought to be between 1:25,000 and 1:50,000 live births.

The first clue to the cause of ZS came from electron microscopic (EM) examination of liver tissue showing an apparent absence of peroxisomes. However, the current first-line investigation of diagnosis of ZS and PZS is the measurement of saturated VLCFAs. The VLCFAs have a chain length of 22 carbon atoms or greater, and in ZS and PZS there are markedly raised ratios of both C24:0/C22:0 and C26:0/C22:0 in all tissues. Additional assessment of peroxisomal function needs to be carried out in atypical cases. Both ZS and PZS are genetically heterogeneous, and 14 complementation groups have been defined using patient fibroblast cell lines. The human genes each of these groups have now been identified (Shimozawa et al. 2005; Wanders and Waterham 2005).

Rhizomelic Chondrodysplasia Punctata

Rhizomelic chondrodysplasia punctata (RCDP) is a peroxisomal disorder genetically and biochemically distinct from ZS. It is characterized by severe proximal limb shortening, early-onset symmetrical cataracts, retinal dysfunction, facial dysmorphisms, ichthyosis, and early lethality. The biochemical hallmarks of RCDP are disordered localization of 3-oxoacyl CoA thiolase and deficiency of plasmalogen biosynthesis. These abnormalities are secondary to the primary deficiency of the PEX7 protein, which is responsible for importing the PTS2 targeting signal proteins into the peroxisome (Braverman et al. 1997; Brites et al. 1998). There is significant phenotypic overlap between RCDP and ZS, suggesting that plasmalogens may have a critical role in, at least, lens development, retinal function, and the synchrony of normal ossification.

Protocol for Postmortem Diagnosis of Inborn Errors of Metabolism

In view of the implications for recurrence risks in future pregnancies within families, it is important to ensure that appropriate samples are stored at postmortem examination of a child where IEM is in the differential diagnosis. Kronick et al. (1983) and Olpin (2004) have suggested protocols for sample collection and storage and these have been amalgamated in Table 7.5.

Conclusion

Genetic biochemical disorders represent some of the most completely understood human genetic disorders and have been indispensable in elucidating normal human biochemical processes. It is important to avoid the trap of believing that there is nothing left to learn. There are likely to be whole new classes of disorders and currently unrecognized presentations of known disorder. Making a biochemical diagnosis in the perinatal period clarifies the genetic risk for subsequent pregnancies, provides a means of accurate prenatal diagnosis, and may allow effective treatment regimens to be instituted. Sir Archibald Garrod pioneered research into IEMs, and his call to "treasure our exceptions" is as important now as it was 90 years ago.

Appendix 7.1: On-Line Information Services

- http://www.ssiem.org.uk/—Society for the Study of Inborn Errors of Metabolism home page, with many useful links
- http://www.bimdg.org.uk/—British Inherited Metabolic Diseases Group home page and United Kingdom Directory of Laboratories Diagnosing Inborn Errors of Metabolism
- http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM—OMIM (On-Line Mendelian Inheritance in Man), a comprehensive catalog of genetic diseases
- http://www.metagene.de/index.html—MetaGene a knowledge-base for inborn errors of metabolism with a searchable interface

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8
Perinatal Hematology

Angela E. Thomas

The perinatal period is one of rapid physiological change, and the distinction between physiological variation and pathological change can become blurred; the extent of prematurity adds further variability. Correct interpretation of hematological abnormalities is dependent on both maternal and fetal history: the health of the mother, complications during pregnancy, placental function, the circumstances of birth, and the gestational and the conceptional age (gestational age and postnatal age) of the infant. For inherited hematological disorders, family history and investigation can also be informative.

Most hematological abnormalities in the neonate have their origin in fetal life, and can arise from the fetus alone, be secondary to maternal pathology, or result from an interaction between the mother and fetus. Maternal pathology often exerts its effects on the fetus via the placenta—the anatomical and physiological link between the fetus and the mother, allowing interaction between the two. The placenta provides the interface between the maternal and fetal circulation, and its integrity and function are of paramount importance to the health of the fetus (Cross 2006). The placenta delivers nutrition and oxygen to the fetus, but acts as a barrier to some classes of antibody and toxins. Immunoglobulin G (IgG) is able to cross the placental barrier, enabling maternal IgG to enter the fetal circulation, giving the fetus some passive immunity against pathogens in the first few months of life. In the same way, harmful IgG antibodies such as anti-D, antiplatelet antibodies, and antiphospholipid antibodies can cross the placenta, causing hematological problems in the fetus. Pathogens, drugs, and toxins may also cross, resulting in intrauterine infection or specific toxic effects such as fetal alcohol syndrome (West and Blake 2005). There are early data to show that diet and toxins may have a small but significant effect on the genetic integrity of the rapidly dividing hemopoietic stem cells, which may be an initiating event for the later development of leukemia or cancer (Sharpe and Franco 1996; Alexander et al. 2001; Thompson et al. 2001). Many hematological abnormalities are associated with placental failure, which, via intrauterine growth restriction (IUGR), can be the final common pathway through which maternal illness or complications during pregnancy affect the fetus. Other abnormalities may arise from complications at the time of delivery such as hemorrhage, or due to acquired factors such as infection or placement of central venous catheters.

Hematological problems are almost universal in premature and sick neonates but can occur unexpectedly in otherwise healthy term babies. Clinical problems often require immediate management and yet the hematological investigations available in most units are comparatively limited. Quantitative tests on peripheral blood and morphological analysis are easily available, but care is needed in taking these samples, which can be technically difficult and subject to artifact or variability, depending on the type of specimen, for example, arterial, venous, or capillary blood samples; the skill with which it was taken; and whether any contaminants, such as heparin, are present. The laboratory must be experienced in

handling small specimens, and correct interpretation requires experience in perinatal hematology, as both numeric values and morphology are highly age dependent. Special and research investigations may give insight into the causes or underlying mechanisms of the problem but are not necessarily widely or rapidly available. They are important for future understanding of the conditions and may lead to new therapies, which currently are rather limited.

Maternal and Fetal Bleeding

The fetoplacental circulation contains around 110 mL/kg of blood (Wardrop and Holland 1995); at 30 weeks' gestation, about 55 mL/kg is in the fetus, rising to 90 mL/kg at term. It follows, therefore, that premature infants will have a lower blood volume, especially if there is early cord clamping, and be more at risk of the consequences of hemorrhage. In many cases, blood loss results in anemia, but it can also result in a reciprocal polycythemia in twin-to-twin transfusion syndrome and in sensitization of the mother to foreign antigens present on fetal blood cells, particularly red cells or platelets. Clinical manifestations depend on the volume and rate of blood loss. If chronic, anemia develops slowly, allowing the fetus to develop hemodynamic compensation. The infant is pale at birth and the blood count shows a microcytic, hypochromic anemia. If the blood loss is severe, cardiac failure and hydrops fetalis may develop (Arcasoy and Gallagher 1995). Acute blood loss prior to delivery leads to an infant who is distressed and hypovolemic; initial blood counts may show a normal hemoglobin that falls rapidly over the first 24 hours of life.

Fetal Blood Loss

Fetomaternal

Fetal blood cells enter the maternal circulation in up to 95% of pregnancies but normally in very small amounts, fewer than 2 mL in 98% of cases (Sebring and Polesky 1990). However, a fetal hemorrhage of ≥30 mL occurs in 0.3% of pregnancies and blood loss of ≥150 mL, causing hemodynamic instability, occurs in approximately 1 in 2800 pregnancies (Sebring and Polesky 1990; Biankin et al. 2003). Survival of fetal red cells in the maternal circulation depends on the blood group antigens on the fetal cells, which may be different from those on maternal cells. Naturally occurring red cell antibodies such as anti-A and anti-B present in maternal plasma bind to and remove incompatible red cells, thus partially protecting the mother from sensitization to other potentially foreign antigens that may be present on fetal cells such as RhD, Rhc, or Kell. However, even ABO-incompatible fetal cells are not always removed rapidly and may survive for weeks in the maternal circulation (Sebring and Polesky 1990), and even if removed rapidly, brief exposure to the foreign antigen can result in a rapid rise in antibody titer. By a similar mechanism, the mother can be sensitized to foreign antigens on the surface of the fetal platelet, which can result in an alloimmune thrombocytopenia in the fetus. The commonest platelet antigen to trigger such a response is the human platelet-specific alloantigen (HPA)-1a.

A fetomaternal hemorrhage can be quantified using the Kleihauer-Betke test, which identifies red cells containing hemoglobin F (HbF) based on the principle that fetal hemoglobin is more stable than the adult form in acidic solutions (Kleihauer et al. 1957; Kelsey et al. 1999). This is important both to ensure that the correct amount of anti-D is given to Rh-negative women at risk of developing anti-D antibodies (Sebring and Polesky 1990; Kelsey et al. 1999) and also to estimate whether the fetus might be at risk from blood loss. A positive test after maternal trauma, regardless of the Rh status of the mother, has been shown to predict the onset of preterm labor (Muench et al. 2004). The manual Kleihauer-Betke test is subjective and imprecise. Automated tests have been introduced that have increased accuracy and sensitivity, allowing detection of fetomaternal hemorrhage in the range of 0.0001% to 1% (Pelikan et al. 2003). Flow cytometry has also been used to estimate fetomaternal hemorrhage, and although not as sensitive as the automated Kleihauer-Betke test, detecting only bleeds of >0.1%, the technique is useful in detecting false positives, which can occur in the presence of maternal persistence of fetal hemoglobin (Kelsey et al. 1999; Nelson 1999; Pelikan et al. 2003, 2004).

Fetomaternal bleeding can result in intrauterine death, and although there is usually pallor of the fetus, autopsy findings can be subtle. It is important, therefore, in investigating an unexplained fetal death, that a Kleihauer-Betke test be performed on the mother (Biankin et al. 2003). It can also be performed on vaginal blood in cases of antepartum hemorrhage to identify a fetal bleed (Akhter et al. 1978; Oyelese et al. 1999).

Twin-to-Twin Transfusion Syndrome

Twin-to-twin transfusion syndrome (TTS) is defined as a difference of hemoglobin concentration of >50g/L and a >20% difference in birth weight between twins. Such differences have been shown in twins without TTS (Wenstrom et al. 1992; Cordero et al. 2005) and can only be ascertained at birth. Ultrasound examination to assess discordant fetal abdominal girth, polyhydramnios/oligohydramnios, transplacental vascular shunting, and differences in umbilical artery velocity are now used antenatally to aid diagnosis (Blickstein 1990). This has the advantage of identifying the problem before delivery, so that intrauterine treatment can be offered. Twin-to-twin transfusion syndrome occurs in monozygotic twins who share a monochorionic placenta and can be chronic or occur acutely before or at birth. When chronic, ultrasound features as described above can be identified before birth. The donor twin develops anemia and oligohydramnios, and the recipient twin develops polycythemia and polyhydramnios. Acute TTS usually results in the donor twin having symptoms of hypovolemia and the recipient suffering acute polycythemia and the risk of hyperviscosity.

Around 60% of monozygotic twins have a monochorionic placenta and 15% to 20% suffer a twin-to-twin transfusion (Lim et al. 2005; Cordero et al. 2006; Rodeck et al. 2006) with a mortality of around 20% (Lim et al. 2005). Different types of anastomoses exist within monochorionic placentas, and they provide the route for blood cell passage (Lopriore et al. 1995). The anastomoses can be asymmetrical, resulting in unequal blood distribution between the twins, with one being polycythemic and the other anemic (see also Chapter 12). Severe TTS is associated with poor neonatal outcome and a relatively high rate of neurological abnormalities (Lopriore et al. 1995; Duncan 2005; Rodeck et al. 2006); cardiac and renal complications may also follow. To improve the outcome in TTS, strategies such as amniotic fluid reduction (Rodeck et al. 2006) or fetoscopic laser coagulation of placental anastomoses have been employed in the more severe cases (Duncan 2005; Lopriore et al. 2006).

Amnioreduction has been associated with a more severe neurological outcome, possibly due to "placental steal," causing a shift of blood from the fetus to the placenta (Duncan 2005; Rodeck et al. 2006). The benefit of laser coagulation over amnioreduction has been demonstrated and is therefore now considered the treatment of choice if the syndrome is severe (Duncan 2005; Yamamoto and Ville 2006). Despite laser therapy, neonatal morbidity and mortality rates remain high when compared with monochorionic twins without TTS, one study showing an adverse neonatal outcome (neonatal death, major neonatal morbidity, or severe cerebral lesions) in 26% versus 8% in those without TTS (Lopriore et al. 2005). Miscarriage, premature rupture of the membranes and preterm delivery may follow laser therapy and account for more than 20% of perinatal mortality after treatment (Yamamoto and Ville 2006). Anastomoses may be missed, with one study showing residual anastomoses in 33% of placentas after delivery (Lopriore et al. 2006). However, in this study there were no differences in adverse outcomes between the infants where the therapy had been complete and those where there were residual anastomoses, although there was an increased incidence of hematological complications, such as a hemoglobin difference of >50g/L, in the latter group.

Intrapartum Blood Loss

In general, fetal hemorrhage is unusual and intrapartum hemorrhage is most commonly of maternal origin. Even when severe, it is almost always manageable, whereas fetal bleeding may be difficult to detect clinically and lead to exsanguination and death (Schmidt et al. 2005). Intrapartum hemorrhage is most commonly due to rupture or tearing of fetal vessels at the time of delivery. Increased risk factors include short cords and velamentous cord insertion. Retroplacental

hemorrhage and placental abruption may lead to significant fetal blood loss. Surgical damage to the placenta may occur at cesarean section, and the placenta may also be injured by maternal abdominal trauma or in a motor vehicle accident.

Neonatal Blood Loss

Internal hemorrhage can occur during a difficult or prolonged labor or with instrumentation such as ventouse or forceps extraction. If the fetus has a hemorrhagic diathesis such as thrombocytopenia or hemophilia, the risk of bleeding is increased. The bleeding tendency may not have been identified before labor, but where it has, special precautions should be in place, such as avoidance of invasive monitoring, prolonged second stage of labor, or instrumental delivery (see below). Recognition of internal hemorrhage may not be immediate, the neonate presenting with shock 24 to 72 hours after birth. Traumatic deliveries may result in subdural or subarachnoid hemorrhages as well as cephalhematomas and subaponeurotic bleeds (see Chapter 13). Cephalhematomas are most common and are limited by periosteal attachments and rarely cause significant clinical problems. Subaponeurotic bleeds, being unchecked by periosteal attachments, can be very large. Exsanguination has been reported (Robinson and Rossiter 1968). Asymptomatic subdural bleeds can occur in up to 6.1% of uncomplicated vaginal deliveries (Doumouchtsis and Arulkumaran 2006). Other areas of bleeding include the retroperitoneum, liver, and spleen; preterm, breech deliveries are at particular risk.

Repeated blood sampling in sick neonates, especially if premature, results in significant blood loss; the realization of this and the use of microtechniques have helped reduce this loss.

Maternal Factors

Intrauterine Growth Restriction

Intrauterine growth restriction is often seen in association with pregnancies complicated by pregnancy-induced hypertension (PIH) or the HELLP syndrome (hypertension, elevated liver enzymes, and low platelets) (Fayyad and Harrington 2005; Duley et al. 2006), chronic hypertension (Mugo et al. 2005; Tamakoshi et al. 2006), maternal diabetes mellitus (Reece et al. 1998; Jaffe 2002; Maulik 2003 Lampl and Jeanty 2004), chronic renal insufficiency (Trevisan et al. 2004), and sickle cell disease. It is also seen with maternal smoking, ingestion of alcohol, and cocaine use (Ashfaq et al. 2003; Vogt Isaksen 2004; Bada et al. 2005; Zdravkovic et al. 2005). Infants with IUGR are distinct from those who are small for gestational age (SGA), although the terms have been used interchangeably (Bamberg and Kalache 2004). The SGA infant is one who has failed to achieve a specific weight, usually defined as the 10th percentile, whereas the IUGR infant has not reached full growth potential due to a pathological process that has occurred in utero. Conditions causing IUGR result in impaired placental function, which effectively renders the fetus malnourished and chronically hypoxic. These babies consequently have specific clinical problems and a characteristic hematological response, whatever the cause of the placental failure.

Hematological Abnormalities in the Growth-Restricted Infant

The hematological abnormalities involve all blood lineages and affect both mature cells and their progenitors. Neutropenia and thrombocytopenia are seen together with large numbers of nucleated red cells with or without polycythemia, indicating increased red cell production. There is good evidence that neutropenia and thrombocytopenia are due to impaired production, although the precise mechanism has not been elucidated (Watts and Roberts 1999). Studies have shown reduced numbers of neutrophil progenitors at birth in babies born to mothers with preeclampsia, along with reduced proliferative and storage neutrophil pools, most marked in babies with IUGR (Koenig and Christensen 1989; Watts and Roberts 1999). Thrombocytopenia has long been associated with IUGR and PIH. These conditions are responsible for about 80% of cases occurring within the first 72 hours in preterm babies (Murray and Roberts 1996). Similarly to the myeloid progenitors, precursors of the megakaryocyte lineage up to

mature megakaryocytes are all markedly reduced at birth (Murray 1999). On the other hand, virtually all babies with IUGR and neutropenia with or without thrombocytopenia show signs of enhanced erythropoiesis with increased numbers of nucleated red blood cells (NRBCs) at levels of >50 NRBCs/100 white cells compared to <20 NRBCs/100 white cells in healthy infants from 24 weeks to term (Watts and Roberts 1999). Polycythemia develops in 25% of babies with IUGR or born to mothers with PIH (Watts and Roberts 1999); there is also evidence of increased erythrocyte production in pregnancies where placental dysfunction has been shown, including pregnancies of women with gestational diabetes (Perrine et al. 1986).

In the growth-restricted baby, increased NRBCs, polycythemia, if present, and neutropenia are most marked at birth and lessen thereafter. The platelet count may be slightly reduced or in the low normal range at birth, reaching a nadir at day 4 or 5. Platelet counts in most babies begin to recover at the end of the first week, attaining normal values by the end of the second week (McIntosh et al. 1988; Koenig and Christensen 1989; Murray and Roberts 1996). Despite this early recovery, some babies develop recurrent cytopenias at times of hemopoietic stress, such as infection in the early neonatal period, suggesting that there is a degree of impairment of hemopoiesis at the stem or progenitor cell level.

The mechanism of these cytopenias has not been fully elucidated but there is some evidence to suggest that the raised erythropoietin levels seen in IUGR babies cause diversion of the as yet uncommitted multipotent stem cells to produce erythrocytes at the expense of other cell lines; so-called lineage steal (McIntosh et al. 1988; Christensen et al. 1989, 1991). It is possible that erythropoietin has inhibitory effects on nonerythroid progenitors, but the evidence is unclear. However, there is some evidence to support production of other inhibitors, possibly from the placenta itself or other factors present in the serum of mothers of babies with IUGR (Koenig and Christensen 1991; Watts and Roberts 1999). Production of cytokines such as granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), or thrombopoietin (Tpo) are not significantly lower in IUGR babies than in healthy term and preterm babies, although levels correlate positively with gestational age (Chirico et al. 1997; Rondini and Chirico 1999; Paul et al. 2002), indicating that the cytopenias are not secondary to reduced or impaired cytokine production. However, Tpo levels do not always increase to the same extent as those in thrombocytopenic children or adults and this reduced response may contribute to the slow platelet recovery and recurrent thrombocytopenia in the postnatal period (Murray et al. 1998; Watts et al. 1999; Paul et al. 2002).

Intrauterine growth restriction also affects other organs, including the spleen, and this is evident on the blood film, with Howell-Jolly bodies present in the red cells, target cells, and spherocytes. The spleen is not the predominant source of hemopoietic cells in the fetus, and therefore the hyposplenism is unlikely to contribute to the neutropenia and thrombocytopenia, but it is important in host defense and its impaired function may contribute to the susceptibility of the IUGR neonate to infection.

Polycythemia is more common in growth-restricted babies than in healthy babies (Tenovuo 1988; Merchant et al. 1992; Werner 1995) and is associated with circulatory changes including reduced cerebral blood flow, increased vascular resistance, and reduced cardiac output. This gives rise to neurological symptoms, cyanosis, tachypnea, tachycardia, and an increased incidence of necrotizing enterocolitis in preterm babies. Biochemical derangements include hypoglycemia and increased bilirubin, the latter due to the increased red cell destruction seen with enhanced erythropoiesis and reduced red cell life span. Treatment with dilutional exchange, probably using crystalloids (Wong et al. 1997), is recommended in symptomatic babies, both term and preterm, with a packed cell volume (PCV) >65%, since resolution of early symptoms and improvement of blood flow has been shown (Watts and Roberts 1999). There are few data on long-term outcome, however. In asymptomatic term babies, observation and symptomatic treatment is recommended, whereas in asymptomatic preterm babies, especially if growth-restricted, exchange transfusion may be appropriate with a PCV >70%.

The neutropenia and hyposplenism seen in infants with IUGR will contribute to the risk of infection, especially if premature. Neonatal neutrophils have reduced function and the immune system is immature. In addition to good infection control measures and prompt and appropriate treatment of presumed or proven infection, the use of recombinant G-CSF is showing some evidence of efficacy in terms of improving neutrophil count (La Gamma et al. 1995; Makhlouf et al. 1995), although whether this translates into reduced infection-related morbidity and mortality is yet to be shown (Calhoun and Christensen 1999).

Thrombocytopenia in infants with IUGR, particularly if the infant is premature, increases the risk of hemorrhagic complications, including intracranial hemorrhage (ICH). Platelet transfusion is the only treatment available, recombinant Tpo as yet having an uncertain role (Haznedaroglu et al. 2002; Kaushansky 2002; Kuter and Begley 2002). The aim with transfusion is to keep the platelet count above 50×10^9/L, although there is often a suboptimal increment (Blanchette et al. 1995), and maintaining a higher platelet count does not necessarily reduce the incidence of ICH (Andrew et al. 1993). However, other studies have shown that thrombocytopenia $<100 \times 10^9$/L is associated with a higher incidence of ICH (Andrew et al. 1987).

Infection

Maternal infection through transplacental spread can cause hematological and other problems in the fetus. They vary depending on the gestational age at which the fetus is exposed. It not always possible to differentiate bacterial from viral or protozoal infection at birth so empirical antibiotics are often used before a definitive diagnosis is made. Infections that are commonly screened for when an infant is born with jaundice, hepatosplenomegaly, thrombocytopenia, or anemia include toxoplasma, rubella, cytomegalovirus (CMV), herpes simplex, human immunodeficiency virus, and syphilis; parvovirus B19 can cause anemia or pancytopenia. Other features such as cataracts, arthralgia, or micrognathia are associated with particular infections. Group B streptococcus is acquired perinatally from the genital tract and can cause infection and acute disseminated intravascular coagulation (DIC) in the neonate.

Immune

Hemolytic Disease of the Newborn

Hemolytic disease of the newborn (HDNB) describes a maternal antibody-mediated fetal hemolytic disease. If the mother is negative for a particular red cell antigen, she will raise antibodies against paternally derived antigens should they be expressed on fetal red cells. The most important group of antigens are the RhD group, although Rhc, RhC, RhE, and Kell, along with Duffy, Lutheran, and other antigens, can also cause severe hemolysis. ABO incompatibility tends to result in mild to moderate HDNB. First exposure to the antigen leads to production of IgM, which does not cross the placenta and is therefore of no consequence to the fetus. However, subsequent exposure stimulates the production of IgG antibodies, which readily cross the placenta and bind to fetal red cells, resulting in their extravascular destruction.

RhD

Exposure to foreign proteins on fetal red cells does not inevitably result in alloimmunization, as immunogenicity depends on host factors and the antigen itself. RhD is the most immunogenic of the red cell antigens, and immunization of the mother generally occurs with a fetomaternal bleed of >0.1 mL. Transplacental hemorrhage is estimated to occur in 75% of pregnancies, most often at delivery, but can also occur following a sensitizing event such as amniocentesis, therapeutic termination of pregnancy, spontaneous abortion, or maternal trauma. The antibody response can be prevented at this stage by the administration of anti-D immunoglobulin, which causes rapid destruction of fetal erythrocytes in the maternal spleen before they are recognized by the maternal immune system as foreign. Assessment of a fetomaternal hemorrhage is an important element in determining the amount of anti-D to be administered to an RhD-negative mother following such a sensitizing event or after delivery of an RhD-positive infant. The presence of fetal red cells in

the maternal circulation can be detected by the Kleihauer-Betke test, and quantitation of the bleed is estimated as described above. The standard dose of anti-D, 500 IU, is sufficient to prevent sensitization from a 4-mL fetomaternal bleed. However, a bleed of ≥30 mL occurs in 0.3% of women (Sebring and Polesky 1990), and therefore protection will not be afforded by the standard dose. For any bleed >4 mL, an appropriate supplementary dose of anti-D immunoglobulin must be given immediately. A repeat estimation of the fetomaternal bleed should be performed 48 hours following the initial dose of anti-D and the plasma screened for anti-D. Where fetal cells are still present or no anti-D is detectable, a further dose of anti-D should be given and monitoring continued every 48 hours until no fetal cells are present and (passive) anti-D is detectable (Kelsey et al. 1999). The incidence of Rh alloimmunization has decreased substantially since the introduction of immune globulin in 1968 (Fantoli 1966; Clarke 1967), although 1% to 2% of RhD-negative women still become sensitized (Royal College of Physicians of Edinburgh/Royal College of Obstetricians and Gynaecologists 1997; Crowther and Keirse 2000), possibly due to passage of small numbers of fetal cells that cross the placenta throughout pregnancy or to insufficient anti-D administration. The incidence can be reduced to about 0.2% by giving prophylactic anti-D to RhD-negative women antenatally, at 28 and 34 weeks' gestation (Crowther and Keirse 2000). Once immunization has occurred, the antibody levels increase with each subsequent pregnancy with an RhD-positive fetus, although antibody level does not accurately predict severity of HDNB (van Dijk et al. 1995). Severe cases may require intrauterine transfusion on one or more occasions to prevent or treat fetal hydrops before the infant can be safely delivered. Blood should be group O or ABO identical with the fetus (if known) and RhD negative; it also should be CMV negative and irradiated (Gibson et al. 2004). Hyporegenerative anemia can follow multiple intrauterine transfusions and may require treatment with erythropoietin in the neonatal period (Scaradavou et al. 1993). Neonatal exchange transfusion may be required to manage severe anemia at birth, particularly in the presence of heart failure, and to treat the severe hyperbilirubinemia that can accompany HDNB.

ABO

ABO incompatibility can also cause HDNB, but clinically significant hemolysis generally only occurs if the fetus is group A and the mother group O, although it can occasionally occur in group B babies. The hemolysis is due to naturally occurring IgG anti-A (or anti-B), but due to the weaker expression of these antigens on neonatal red cells, clinically significant hemolysis is uncommon. The diagnosis is not straightforward since the degree of hemolysis is not directly related to level of maternal antibody, and although the most severely affected babies will have a positive direct antiglobulin test (DAT), this is not always the case. Thus the diagnosis of ABO HDNB is a diagnosis of exclusion, with the constellation of signs of a falling hemoglobin, a rising bilirubin, and ABO incompatibility with the mother with or without a positive DAT in the absence of any other alloantibodies (Gibson et al. 2004). Spherocytes are a prominent feature of the blood smear (Fig. 8.1). If transfusion is needed, group O blood compatible with maternal plasma should be used since adult cells of the baby's own group will express higher levels of A or B antigen and may cause increased hemolysis.

Kell

Anti-Kell antibodies occur in around 0.1% of pregnant women (Mayne et al. 1990) and the problems that result are different from those in Rh or ABO incompatibility. This is due to the

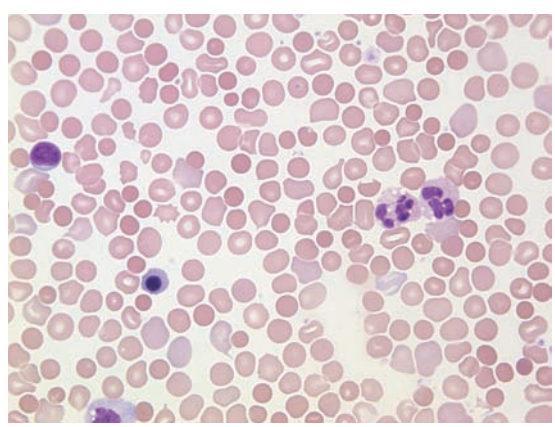

Figure 8.1. Spherocytes and nucleated red cells in a neonate with hemolysis secondary to ABO-incompatibility.

early expression on the erythrocyte of the Kell 1 antigen, or its antithetical variant Kell 2, when the erythrocyte is at its peak proliferative capacity. Reticulocyte count, nucleated red cell count, and bilirubin are all lower when compared to fetuses with RhD HDNB (Vaughan et al. 1994), suggesting that in maternal Kell alloimmunization erythroid suppression rather than hemolysis is the predominant mechanism producing fetal anemia. Amniotic fluid bilirubin concentrations correlate poorly with the anemia, and the severity of the disease does not correlate with the antibody titer (Leggat et al. 1991). Fetal blood sampling is the method of choice for monitoring a severely affected fetus (Mayne et al. 1990; Leggat et al. 1991; Vaughan et al. 1994). Intrauterine transfusions may be needed, as well as top-up transfusions in the neonatal period or phototherapy. In two retrospective studies, the rate of stillbirth in affected babies was around 20% (Mayne et al. 1990; Leggat et al. 1991).

Neonatal Alloimmune Thrombocytopenia

Neonatal alloimmune thrombocytopenia (NAIT) arises from an incompatibility of the human platelet-specific alloantigen (HPA) epitopes between the pregnant mother and her baby. The frequency of NAIT is estimated at 1 in 1500 to 1 in 5000 live births (Kaplan et al. 1992; Uhrynowska et al. 2000; Turner et al. 2005), but this is probably an underestimate. Turner et al. (2005) conducted a prospective study using antenatal screening to detect NAIT. Of 26,506 women, 318 (1.2%) had anti–HPA-1a antibodies and the incidence of babies born with NAIT was 1 in 2300. Five babies (1 in 3700) had severe thrombocytopenia; three had mild bleeding symptoms and there were no intracranial hemorrhages or intrauterine deaths. Serious bleeding in NAIT due to anti–HPA-1a is likely to be overreported in the literature in the absence of prospective studies, with the risks of serious morbidity and mortality being reported as high as 20% and 10% respectively (Kaplan et al. 1992).

Over 80% of severe cases of NAIT in the Caucasian population are due to differences in the presence of HPA-1a epitope, with the mother being negative and the fetus positive. Development of anti–HPA-1a antibodies occurs in 10% of such cases and these antibodies, being IgG, can cross the placenta and cause NAIT in the fetus or neonate (Bessos and Seghatchian 2005).

The second most common cause of NAIT is antibody production to the HPA-5b epitope, accounting for 4.3% of cases (Campbell-Lee et al. 2003), but other epitopes have been implicated, including HPA-15 and the human leukocyte antigen (HLA) system (Panzer et al. 1995; Han et al. 2002; Ertel et al. 2005; Mandelbaum et al. 2005; Thude et al. 2006). The HLA antibodies are common in the pregnant population and can interfere with platelet transfusions in NAIT (Panzer et al. 1995; Grainger et al. 2002). Anti–HPA-1a can cause severe problems in the first pregnancy, whereas the others tend to only cause problems in the second or subsequent pregnancies if at all (Panzer et al. 1995; Bessos and Seghatchian 2005). Clinical manifestations can vary from mild, such as petechiae and bruises, to severe, including death; intracranial hemorrhage, 50% of which occur *in utero* (Johnson et al. 1997); and significant neurological sequelae. Although anti–HLA-1a antibodies bind to the IIb-IIIa glycoprotein receptor on the platelet surface (Beadling et al. 1995; Bessos et al. 2003; Sukati et al. 2005), they do not seem to interfere with fibrinogen binding (Beadling et al. 1995) but may give rise to a more severe phenotype, as HPA-1a has a higher number of antigenic sites per platelet than HPA-5B (Campbell-Lee et al. 2003). In general, the level of maternal antiplatelet antibody has not been predictive of the degree of thrombocytopenia (Kaplan et al. 1988; Panzer et al. 1995; Bessos et al. 2005), which is strongly related to a previous history of affected offspring (Johnson et al. 1997), although some groups have found an association (Williamson et al. 1998; Jaegtvik et al. 2000).

To determine the degree of thrombocytopenia in a fetus at risk of NAIT, fetal blood sampling can be performed and intrauterine platelet transfusions given (Kaplan et al. 1988; Lipitz et al. 1992). Intrauterine transfusion is now considered the optimal treatment for NAIT, and accredited panels of donors negative for HPA-1a or HPA-5b antigens are being established (Allen et al. 2004). These panels of donors can also be used to provide HPA-1a/HPA-5b negative apheresis platelets promptly for a neonate diagnosed with NAIT. Compatible platelets should be available at the

time of fetal sampling for NAIT, even if the primary purpose is not that of transfusion, because in the presence of severe fetal thrombocytopenia, hemorrhage can be prevented by platelet transfusion. The platelets should be group O, RhD negative, or compatible with maternal antibody, and should be HPA compatible with maternal antibody. They should preferably be collected by apheresis and should be irradiated. If intrauterine platelet transfusion is not possible, maternal intravenous IgG is an alternative and has been shown to reduce the risk of intracranial hemorrhage even when thrombocytopenia persists (Lipitz et al. 1992; Radder et al. 2004). Compatible platelet transfusions should be given to neonates identified as having NAIT with platelet counts $<20 \times 10^9$/L if clinically well or $<50 \times 10^9$/L if there has been an intracranial hemorrhage or other serious bleed. Platelets should continue to be irradiated if intrauterine platelet transfusions have been given. If no compatible platelets are available, intravenous immunoglobulin and random pools of platelets can be used.

Maternal Idiopathic Thrombocytopenic Purpura

Idiopathic thrombocytopenic purpura (ITP) in pregnancy is about 100 times less common than benign gestational thrombocytopenia, which occurs in about 7% of pregnancies (Allford et al. 2003) but can be difficult to differentiate from it. Thrombocytopenia may also be an indication of preeclampsia, coagulopathy, or autoimmune disease and pregnant women with platelet counts $<100 \times 10^9$/L should be screened for these conditions and the diagnosis of ITP made as one of exclusion. Although in ITP there may be transmission of antiplatelet antibodies to the fetus with possible fetal or neonatal thrombocytopenia, significant clinical sequelae are rare. Large prospective studies showed an incidence of severe thrombocytopenia with platelets $<50 \times 10^9$/L in 8.9% to 14.7%, with intracranial hemorrhage occurring in 0% to 1.5% of infants (Bussel et al. 1991; Burrows and Kelton 1993). A study by Fujimura et al. (2002) showed that of 286 neonates, 22.4% had thrombocytopenia but only 6.3% had bleeding, none of which was severe. The mother should be treated on clinical grounds, as there are no data to show that interventions affect fetal or neonatal platelet count (Burrows and Kelton 1990b; Fujimura et al. 2002; Allford et al. 2003), and delivery should be dictated by obstetric considerations; most hemorrhagic events in the neonate occur 24 to 48 hour after delivery, at the nadir of the platelet count.

Antenatal determination of the fetal platelet count by cordocentesis or fetal scalp blood sampling has been abandoned due to problems and complications with the sampling (Burrows and Kelton 1990b). Following delivery, a cord blood should be taken and those infants with thrombocytopenia closely monitored. Treatment is rarely needed but intravenous (IV) IgG produces a rapid response in infants who are bleeding or have platelet counts $<20 \times 10^9$/L. Life-threatening bleeding should be treated by IV IgG and platelet transfusion (Burrows and Kelton 1992). Since severe thrombocytopenia and bleeding are unusual in neonates of mothers with ITP, NAIT should be excluded by laboratory testing. This is important for both management of the neonate and management of future pregnancies (Burrows and Kelton 1990a).

Maternal Systemic Lupus Erythematosus

Rheumatic autoimmune diseases have a higher prevalence in women, particularly during the child-bearing years. Autoantibodies in the mother, if of IgG isotype, will cross the placenta and may cause problems in the fetus or neonate. While congenital heart block and skin manifestations are the commonest manifestations of this disease in the neonate, hematological problems can occur (Watson et al. 1988; Tseng and Buyon 1997; Weston et al. 1999). Thrombocytopenia has been reported, both as an immune phenomenon (Watson et al. 1988; Weston et al. 1999) and in association with microvascular hemolysis (Hariharan et al. 2000).

Maternal Antiphospholipid Syndrome

Antiphospholipid syndrome (APLS) in pregnancy has been associated with placental insufficiency leading to recurrent fetal loss, preeclampsia, and

fetal growth retardation. Antiphospholipid antibodies (APAs) may cause placental thrombosis, trophoblast dysfunction, and maternal hormonal imbalance leading to pregnancy loss (Heilmann et al. 2003). The exact pathogenesis of the vasculopathy remains unknown. Antiphospholipid antibodies also inhibit trophoblast proliferation, probably through binding to phosphatidylserine, which is externalized during differentiation, leading to reduced trophoblast cell invasiveness, which is a characteristic of preeclampsia and IUGR (Heilmann et al. 2003). The incidence of IUGR in pregnant women with APAs is between 15% and 24% (Polzin et al. 1991; Kutteh et al. 1999) and the clinical features and management of IUGR have been described above. In addition to thrombocytopenia in the infant, thrombosis has occasionally been reported (Sheridan-Pereira et al. 1988). Although maternal treatment with low molecular weight heparin and aspirin improves pregnancy outcomes, there is still a 30% failure rate, and fetal growth restriction, gestational hypertension, and premature delivery are frequent complications (Greaves et al. 2000; Heilmann et al. 2003).

Deficiencies, Drugs, and Toxins

Deficiencies

Maternal vitamin B_{12} deficiency can result in deficiency in the fetus and presentation with cytopenias, failure to thrive, and seizures (Rosenblatt and Whitehead 1999). Treatment with vitamin B_{12} results in rapid resolution. Maternal folate or iron deficiency can lead to premature or low-birthweight infants (Rosenblatt and Whitehead 1999; Hindmarsh et al. 2000), but neither is particularly associated with hematological abnormalities in the neonate.

Drugs

Many drugs have potential effects on the fetus and are often contraindicated in pregnancy. Of particular note are those interfering with vitamin K metabolism, including warfarin, anticonvulsants, rifampicin, and isoniazid (see below). Others include cytotoxics, which may cause pancytopenia and steroids, often given at onset of premature labor to help fetal lung maturation, which can cause a neutrophilia in the neonate.

Toxins

Toxins such as nicotine, cocaine, and alcohol tend to exert their effects through placental failure and have been discussed briefly above. Other toxins have different effects, possibly through damage to DNA.

Infant and Childhood Leukemia

Infant leukemia by definition occurs in children up to the age of 1 year and can present at birth or in the neonate. It has been established that the chromosomal translocation resulting in the leukemia is nearly always a prenatal event (Greaves et al. 2003), and that other chromosomal translocations occurring in children with both acute lymphoblastic and acute myeloid leukemia may also occur in the prenatal period (Wiemels et al. 2002; Greaves et al. 2003). While these events may occur "in error" during rapid hematological proliferation, a study by Thompson et al. (2001) provides evidence that folate supplementation during pregnancy protects the child from acute lymphoblastic leukemia, and an international case-control study found a significant and selective association with infant leukemias that had the specific *MLL* translocation associated with infant leukemia either with exposure to pesticides during pregnancy, especially propoxur, or with consumption of the drug dipyrone (Alexander et al. 2001).

Fetal and Neonatal Factors

Fetal and Neonatal Coagulopathy

The hemostatic system in the fetus and neonate is different from that of the infant or child. Although immature by adult standards, it is physiologically adapted for birth and matures rapidly over the first 6 months of life when adult values are achieved for most hemostatic parameters apart from fibrinogen level and platelet number, which are age independent. However, in sick and preterm neonates, not only is the

system less mature, but acquired coagulopathies place an additional burden on its integrity and ability to compensate.

Investigation

It is essential when interpreting tests of hemostasis that gestational and postnatal age-dependent ranges are used. Ideally, each laboratory should develop its own series of ranges, as results are dependent on instrumentation, reagents, and test methodology. However, due to the inherent difficulties of this approach, ranges are often based on data from the literature (Williams et al. 2002). It is also important to ensure that blood for assay is taken without contamination, especially by heparin, which is often used to keep lines patent and prolongs the clotting tests, and without activation of the coagulation system, which shortens the tests and may cause spurious thrombocytopenia due to platelet clumping. Direct venepuncture is ideal, but in practice, blood is often taken through plastic lines and artifact should be considered in interpretation of abnormal tests. The screening tests used in initial investigation of a neonate who is bleeding, sick, or about to undergo a surgical procedure include platelet number, prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen level or thrombin clotting time (TCT). For neonates with a family history of a bleeding disorder, or those with a characteristic presentation, for example, purpura fulminans, specific factors may be checked in addition. Further tests are dependent on results and clinical state.

Acquired Defects

The vast majority of defects are acquired, and the two most common are vitamin K deficiency bleeding (VKDB), previously termed hemorrhagic disease of the newborn, and disseminated intravascular coagulation (DIC). Neonates are relatively deficient in vitamin K and those who do not receive supplements are at risk of serious bleeds including intracranial hemorrhage. It is recommended that all newborn babies should receive prophylactic vitamin K to prevent such bleeding (Department of Health 1998). Levels of the vitamin K–dependent coagulation proteins, which include the procoagulant factors II (prothrombin), VII, IX, and X, and the anticoagulant factors protein C and protein S are physiologically low at birth and require vitamin K to be active. The bleeding has been described as early, classical, or late, and deficiency is suspected initially by a prolongation of the PT followed by prolongation of the APTT as the deficiency progresses. Early cases occur where the mother has been on anticonvulsants or other drug that affect vitamin K metabolism, and oral vitamin K has been recommended for such mothers in the last 4 weeks of their pregnancy in addition to routine prophylaxis (Delgado-Escueta and Janz 1992). Classical VKDB, occurring 2 to 5 days after birth, can be prevented by intramuscular or oral vitamin K at birth. The intramuscular route will also protect the baby from late VKDB, which occurs after 1 month, but if given orally, repeated dosing is necessary to a total of three doses in breast-fed infants who are at particular risk, as breast milk contains very little vitamin K, whereas formula milk is fortified. If VKDB should occur, treatment is with slow intravenous, or subcutaneous but not intramuscular, vitamin K with factor support given as fresh frozen plasma if the infant is bleeding or possibly prothrombin complex concentrate if the bleeding is intracranial or life threatening (Williams et al. 2002).

Disseminated intravascular coagulation describes a process where both coagulation and fibrinolysis are activated in an uncontrolled way and results in consumption of clotting factors, leading to bleeding, and anticoagulants, leading to thrombosis in small vessels and end-organ damage. The clinical spectrum ranges from compensated low-grade DIC to fulminant DIC. It occurs in the neonate, especially if premature, secondary to a variety of insults, including infection, respiratory distress, metabolic disturbances, and in association with maternal or obstetric disorders such as severe preeclampsia, placental abruption, and in utero death of a co-twin. It is characterized by a prolonged PT, initially a shortened and then lengthening APTT, and falling levels of fibrinogen and platelets. Serial testing may clarify the process, as values of fibrinogen and platelet number may initially be normal and fall with time. The most important aspect of management is to treat or remove the underlying cause, correct acidosis, and maintain tissue perfusion. Support for bleeding may include fresh

frozen plasma (FFP), cryoprecipitate, and platelets; antithrombotic agents such as heparin, protein C, or antithrombin concentrate are not routinely recommended (Williams et al. 2002).

Thrombocytopenia

Thrombocytopenia, too, is most commonly acquired and may be fetal, early onset (occurring within the first 72 hours of birth), or late onset. Causes of fetal thrombocytopenia include NAIT; other immune phenomena such as maternal ITP, APLS, or systemic lupus erythematosus (SLE); congenital infections (see below); aneuploidy; severe Rh hemolytic disease; or other congenital disorders such as Wiskott-Aldrich syndrome. Early-onset thrombocytopenia is seen with placental insufficiency, which accounts for <80% of cases (Watts and Roberts 1999), and is associated with maternal hypertension and diabetes, and IUGR as discussed above. The other main cause is infection, such as with group B streptococcus and DIC, which account for 10% to 15% of cases. Perinatal asphyxia, immune phenomena (the immune causes of fetal thrombocytopenia can result in a low normal count at birth, which falls over the first few days), and thrombocytopenia in association with a microangiopathic hemolytic anemia account for the majority of the others. Late-onset thrombocytopenia is most commonly seen with sepsis (90%) and in association with necrotizing enterocolitis (Watts and Roberts 1999). The fall in platelet count may be the first indication of the underlying condition, and the thrombocytopenia is usually severe, often associated with DIC, and requires multiple platelet transfusions. Although recommendations are not based on randomized controlled trials, it is generally accepted that all neonates with platelet counts of $<20 \times 10^9$/L should be transfused, and in premature or sick neonates platelets should be kept $>30 \times 10^9$/L (Gibson et al. 2004). In the event of major hemorrhage, platelets should be kept $>50 \times 10^9$/L (Murray et al. 2002).

Thrombosis

Although neonates and infants account for the largest number of thrombotic events seen in the pediatric population, these events are still uncommon (Andrew et al. 1994). They are seen most frequently in sick and preterm infants, who may have acquired deficiencies of protein C and protein S, and are most often associated with the presence of an indwelling catheter. Other contributory factors include sepsis, asphyxia, dehydration, maternal diabetes, and cardiac disease. Renal vein thrombosis may occur spontaneously, usually presents within the first few days of birth, and is not necessarily associated with a prothrombotic defect. The impact of such defects on the risk of neonatal thrombosis is not yet clear but thromboses are very rare in healthy babies. Diagnosis is by Doppler ultrasound, but contrast angiography may be needed for the upper limbs in particular, and computed tomography (CT) or magnetic resonance imaging (MRI) scans may be needed for the central nervous system. Treatment can be conservative, such as removal of the catheter with supportive therapy, which includes monitoring for extension of the clot; anticoagulants such as heparin can be used, and thrombolytic therapy if the thrombosis is organ or limb threatening (Williams et al. 2002).

Congenital Defects

Hemophilia A (factor VIII deficiency) is the commonest of the inherited coagulation factor deficiencies followed by hemophilia B (factor IX deficiency), and both can present in the neonatal period. If a family history is known, pregnancy and delivery should be managed among the obstetric, neonatal, and hematology teams to reduce the bleeding risk for both the mother and baby; guidance is available for the management of such women (Kadir 1999). The greatest risk of bleeding is at the time of birth. This can be reduced by avoiding instrumental delivery or vacuum extraction, spontaneous delivery or elective cesarean section being the preferred options. Invasive fetal monitoring should also be avoided (Thomas and Chalmers 2005). Although this advice is most pertinent to boys, it should apply to girls too, as occasionally, if carriers, they can have significantly low factor levels. Once the baby is born, a cord blood sample should be sent for the appropriate factor assay and ideally confirmed later on a peripheral sample; vitamin K injection should be withheld until the results are known or given orally. Severe and moderate hemophilia A and B can be diagnosed at birth; the mild forms may be

more difficult to confirm since factor VIII may be raised into the normal range due to its acute-phase reactant activity, and the levels found in mild factor IX deficiency overlap with the normal range at birth (Williams et al. 2002). The majority of bleeds occur within the first week and may manifest as oozing from puncture sites, hematomas secondary to intramuscular vitamin K, and extracranial or intracranial bleeds; umbilical stump bleeding is rare. The risk of intracranial hemorrhage in a neonate with hemophilia A or B is about 3.5% (Kulkarni and Lusher 1999), most presenting on day 4 or 5, which is important to remember in facilities where babies are discharged at 24 hours. Early routine cranial ultrasound may miss bleeds but should be performed if there has been a traumatic birth or there is clinical suspicion. If negative but clinical suspicion remains, the baby should proceed to a CT or MRI scan. Prophylactic factor replacement has been suggested in all babies with a diagnosis of hemophilia at birth (Buchanan 1999), but early treatment has been associated with an increased incidence of inhibitor formation and therefore cannot be routinely recommended at present (Lorenzo et al. 2001; Van der Bom et al. 2003). Well babies who present with unexpected bleeding, especially if they are term, should be investigated for an inherited bleeding disorder. A comprehensive review of rarer coagulation disorders, including thrombotic problems are covered in a British Committee for Standards in Haematology (BCSH) guideline (Williams et al. 2002).

Purpura Fulminans

This rare disorder, which usually presents within a few days of birth, is due to homozygous protein C deficiency or compound heterozygous protein C and protein S deficiency. Thromboses in the microcirculation are characteristic, giving the clinical picture of purpura fulminans with renal failure and neurological dysfunction. The coagulation tests are compatible with DIC, and measurement of protein C (or S) shows very low or absent levels. However, these proteins are physiologically low at birth, so only if undetectable levels are found is the diagnosis certain. Parents should be heterozygous and show low protein levels. Treatment is with protein C concentrate if available or FFP, which is also used for protein S deficiency where there is no concentrate (Williams et al. 2002).

Congenital thrombocytopenia is seen in Wiskott-Aldrich syndrome, thrombocytopenia with absent radii (TAR) syndrome, Fanconi's anemia, Bernard-Soulier syndrome, and the May-Hegglin anomaly. Clinical or morphological features may help with the diagnosis of these conditions.

Microangiopathic Hemolytic Anemia

Microangiopathic hemolytic anemia is the intravascular destruction of red cells that occurs either due to an abnormal microcirculation or due to low or absent levels of the von Willebrand Factor (VWF) cleaving protease ADAMTS-13 (Veyradier et al. 2003; Tamakoshi et al. 2006). The abnormal microcirculation or increased levels of high molecular weight VWF found in ADAMTS-13 deficiency result in platelet activation, formation of platelet thrombi, and shearing of red cells in the abnormal blood flow that results. The blood count typically shows an anemia and thrombocytopenia with fragmented red cells and polychromasia seen on the film (Fig. 8.2). Hemoglobinuria, a raised bilirubin, and lactate dehydrogenase will be found, and if the process is severe, a coagulopathy with low fibrinogen levels may occur. Kasabach-Merritt syndrome is the association of giant cavernous hemangiomas and DIC. These hemangiomas usually present at birth, grow in size over the first

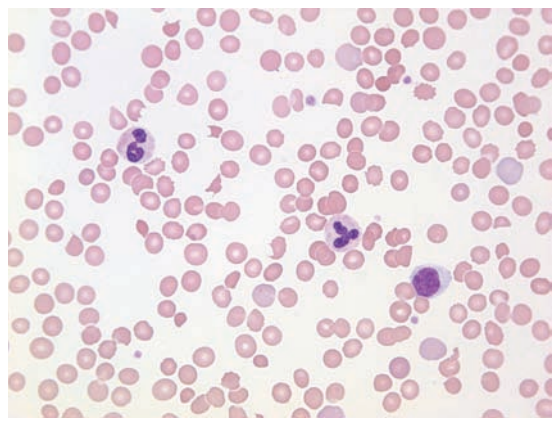

FIGURE 8.2. Microangiopathic hemolytic anemia. Blood film showing fragmented red cells, polychromasia, and reduced numbers of large platelets.

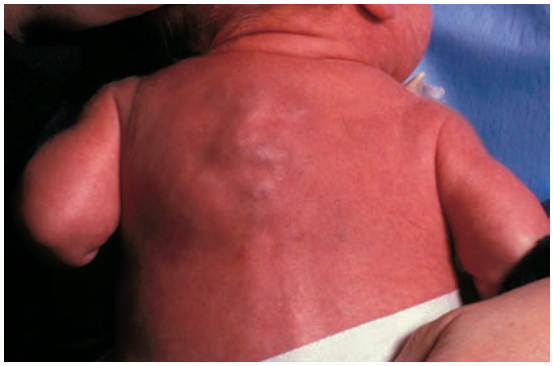

Figure 8.3. A day old neonate with an extensive hemangioma.

few months, and then gradually recede (Fig. 8.3). Sometimes the hemangiomas are visceral and therefore hidden, suspected only due to the hematological abnormalities. Computed tomography or MRI may be helpful in these cases, particularly as there is a high mortality rate (Enjolras et al. 1990). ADAMTS-13 deficiency has been successfully treated by recurrent infusion of FFP (Scully et al. 2006).

Inherited Anemia

Inherited anemias can be due to decreased production as in the rare congenital dyserythropoietic anemias, congenital sideroblastic anemia, or Diamond-Blackfan anemia, which is inherited in 10% to 20% of cases. Increased destruction is seen in red cell membrane disorders such as hereditary spherocytosis and elliptocytosis, both of which have distinctive blood film appearances, although they may be more difficult to interpret in the neonate where spherocytes and poikilocytes are more commonly seen. Defects of hemoglobin synthesis also result in a shortened half-life of the red cell and can be divided into the thalassemias, characterized by globin chain production imbalance, and the hemoglobinopathies, such as sickle cell disease, caused by amino acid substitution.

Thalassemias result from failure to synthesize α or β chains of HbA ($\alpha_2\beta_2$) and HbF ($\alpha_2\gamma_2$). The most severe form of α-thalassemia, where all four α genes are absent, leads to hydrops and death in utero, as no functional hemoglobin can be made in the later stages of gestation (Fig. 8.4). Absence of one to three α genes is compatible with life, with only the three-gene deletion having a significant clinical effect. β-thalassemia affects only HbA and therefore does not affect the fetus or neonate to any significant degree. Likewise amino acid substitutions affecting the β chains such as occur in sickle cell disease have minimal affect on the fetus or neonate. The defects Hb synthesis can be diagnosed both in their homozygous and heterozygous forms using high-performance liquid chromatography, which has largely replaced hemoglobin electrophoresis. It is more sensitive and specific and is able to detect small amounts of normal or abnormal hemoglobins. This is helpful in the neonatal setting, as HbA levels are low and β-globin chain variants or deficiencies are therefore more difficult to detect. In England, an antenatal and neonatal National Health Service sickle and thalassemia screening program began in 2004.

Neonatal anemia can be due to red cell enzyme deficiencies or syndromes with chromosome instability such as Fanconi's anemia or dyskeratosis congenita.

Down Syndrome

Children with Down syndrome (DS) have a 10- to 20-fold increased risk of developing leukemia and a 500-fold risk of acute megakaryoblastic leukemia (AMKL) developing, which occurs during the first 4 years of life and may present in the neonatal period (Zipursky et al. 1992; Ahmed et al. 2004). Of neonates with DS, 10% to 20% develop

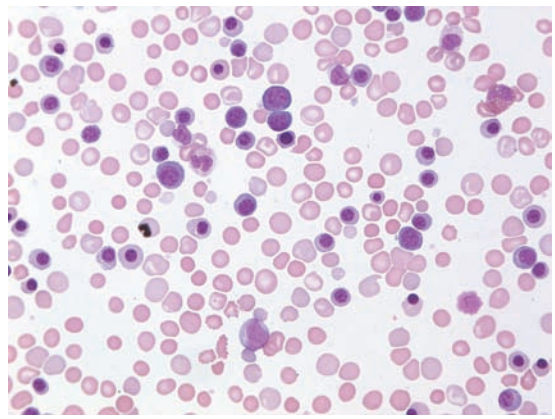

Figure 8.4. Blood film from a fetus with hydrops fetalis showing numerous nucleated red cells and polychromasia.

transient abnormal myelopoiesis (TAM), which may be clinically silent and noted incidentally or associated with clinical features such as hepatosplenomegaly and jaundice. The blood film shows circulating megakaryoblasts but other cell counts may be normal. In most cases, TAM resolves spontaneously, but in up to 30% of cases AMKL develops. Transient abnormal myelopoiesis s a clonal disorder, and mutations in the megakaryocytic transcription factor GATA1 have been shown (Ahmed et al. 2004). Mutations have also been found in blood from neonatal blood spots of asymptomatic children with DS as well as in the blood of those who have developed AMLK. These mutations have not been found in non-DS cord blood samples (Ahmed et al. 2004) or in children with AMLK who do not have DS. The leukemia responds to modified standard therapy for acute myeloid leukemia.

Leukemoid Reaction

Leukemoid reactions can be myeloid or lymphoid and are generally applied to reactions where the cell line in question is $>20 \times 10^9$/L and circulating immature cells are seen. They are generally seen in response to bacterial or viral infection and may be confused with a leukoerythroblastic blood film but distinguishable, as NRBCs are not present in leukemoid reactions and the white cell count is often normal in a leukoerythroblastic film (Bain 1995).

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9
Epidemiology of Fetal and Neonatal Death

Jean Golding

The study of perinatal mortality rates has become increasingly popular. The rates are quoted variously by politicians, sociologists, and clinicians to support whatever point they wish to make. Government spokesmen quote a fall in the perinatal mortality rate as vindication of their policies. Politicians in opposition and clinicians quote high national rates in comparison with selected other countries and claim that the government is not spending enough on maternity or neonatal services. Sociologists point to the differential mortality between advantaged and disadvantaged social groups and claim that better housing, or increased minimum wages, or decreased levels of unemployment would result in an improvement in perinatal mortality rates.

Are any of these claims justified? To interpret any data successfully, whether they relate to a small area or a large country, it is essential to understand the difficulties inherent in statistics. On the face of it, there should be no difficulty in ascertaining the perinatal mortality of a particular population. The only items of information necessary are the absolute numbers of total births and perinatal deaths. However, there are two major problems: first, the definition, and second, the method of recording.

Accuracy of Perinatal Death Statistics

Definition Problems

There are no problems in identifying as live births infants who are born alive and survive well into the first week of life. However, there has been inconsistency in defining those live births that survive for only a short time. To this end, therefore, the World Health Organization (WHO) (1967, 1977) defined a live birth as "the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy, which after such separation, breathes or shows any evidence of life such as beating of the heart, pulsation of the umbilical cord or definite movement of voluntary muscles, whether or not the umbilical cord has been cut, or the placenta is attached: each product of such a birth is considered liveborn." As some very early abortions show such signs, the identification of some live births depends on the assiduousness with which the attending clinician observes and records. This has been demonstrated recently when apparent increases in neonatal death rates over time were shown to be related to changes in the registration of live births weighing <500 g (Joseph and Kramer 1996; Phelan et al. 1998).

A definition of a fetal death is consequently as difficult as that of live birth in an infant showing marginal signs of life. Perhaps more importantly, there is a difference between and within countries concerning which fetal deaths should be registered. Thus, in Japan, all fetal deaths occurring after 13 weeks' gestation are registered (Kamimura 1976), whereas in the United Kingdom only those occurring from 24 weeks have been recorded since October 1992 (Alberman et al. 1997), prior to which only those delivered at or after 28 weeks were recorded (Macfarlane and Mugford 1984). Even so, there is still some confusion concerning neonatal death and late fetal death (stillbirth). In

some countries a live birth that dies before registration as liveborn is registered as a fetal death. Within countries, particular factors may also influence whether a death is registered as a late fetal death or a neonatal death. These include the laws governing the entitlement to a maternity grant. In other countries, some neonatal deaths are deliberately not counted. For example, in the former Soviet Union if a live birth died within 7 days and was less than 1000 g or 28 weeks, then it was not counted in the national statistics at all (Gourbin and Masuy-Stroobant 1995).

Finally, assuming that fetal deaths, live births, and neonatal deaths have been registered, what should the definition of a perinatal death be? The World Health Organization (1977) recommends that for *national* statistics, the term *perinatal death* should include all early neonatal deaths together with those fetal deaths that weigh 500 g or more (or, if weight is unknown, of 22 weeks' gestation or longer, or crown–heel length of 25 cm or longer). For *international* statistics, the WHO recommends including only those fetal deaths of weight 1000 g or more (or, if weight is unknown, of 28 weeks' gestation or longer, or crown–heel length of 35 cm or longer). This weight criteria can make a considerable difference to the results; for example, a study in Belgium showed that the criteria of 500 g resulted in a perinatal mortality rate of 15.1 per 1000 compared with 10.2 per 1000 when 1000 g was used (de Wals et al. 1989). There has been much discussion concerning the bias generated by taking a definition for international comparison that is based on birthweight rather than gestation (Goldstein and Butler 1977; Meyer 1977). Nevertheless, because gestation is often unknown, it is felt that a criterion based on weight will yield more comparable figures than would have pertained had gestation been used.

Personal Variation in Interpretation of Definitions

An elegant study was carried out by Keirse (1984) in Belgium and the Netherlands. He sent to specialist and trainee obstetricians in the two countries a questionnaire purporting to be concerned with the management of preterm labor. It included three case histories, and the respondents were asked whether they would or would not register the deaths. The three deaths had the following characteristics: (1) normally formed, 23 weeks, 590 g, live birth, died at age 4 minutes; (2) anencephalic, 24 weeks, 600 g, died after a few minutes; (3) anencephalic, 24 weeks, 600 g, stillborn. In both countries, by law, cases 1 and 2 should have been registered, but not case and 3. Only 6% of obstetricians responded correctly! No similar studies have been carried out in other countries, but studies of this nature are an essential prerequisite in assessing the validity of published perinatal mortality data.

Registration Failure

Much variation in registration also occurs if a delay is allowed before the death is registered; the longer the legal limit, the less likely are still births and early neonatal deaths ever to be recorded in the vital statistics (Gourbin and Masuy-Stroobant 1995). Although clinicians may fail to recognize when a perinatal death should be registered, even when they fill in forms appropriately these may not reach the registrar. A prime example of this type of error was found in the state of Georgia. Interest was aroused by a peculiar pattern of birth-weight-specific mortality rates. A study was carried out to ascertain how many of the registered live births of low birth weight had indeed survived. It was found that a large proportion (21%) of neonatal deaths had never been registered as such. Their inclusion in the mortality rates would have raised the overall neonatal mortality rate by 25% (Allen and Terry 1979; McCarthy et al. 1980). As a result of this study, all hospitals in Georgia now report all in-hospital deaths, and if registration forms are not received, steps are taken to obtain them. For fetal deaths it has been reported from Wisconsin that 18% were not registered, the shortfall being particularly those of shortest gestation (Greb et al. 1987).

Elsewhere it has been shown that there are similar problems. For example, in Enschede in the Netherlands, Smits (1981) found that 31% of perinatal deaths had not been registered. In Amsterdam hospitals Doornbos and Nordbeck (1985) found that 15% of deaths were not registered, but they were not able to estimate what the rate of

home deaths might be. In Northern Ireland, Scott and colleagues (1981) suggested that there was a 10% shortfall. In the Greek National Birth Survey (Tzoumaka-Bakoula 1990), 31% of the perinatal deaths had not been registered, and in the Jamaican Perinatal Mortality Survey the proportion was over 90% (McCaw-Binns et al. 1996). In Taiwan, a study of all births over a 3-day period showed a true neonatal mortality rate of 6.7 per 1000 births compared with 1.9 registered (Chen et al. 1998).

It is essential that each study of perinatal deaths starts by ascertaining the defects in the data. In local hospital-based studies, for example, one would need to be aware of transfers to other hospitals (or home) and the outcome of each before publishing a neonatal or perinatal mortality rate. In the U.K., national birth surveys, to a certain extent, have provided information that enables us to state that a large majority of perinatal deaths are, in fact, registered as such (Butler and Alberman 1969; Chamberlain et al. 1975); this is likely to be the consequence of a dual system of compulsory birth notification within 7 days by the maternity care provider and a longer period for registration by the family (Gourbin and Masuy-Stroobant, 1995).

Epidemiological Analyses

Given all the problems of definition outlined above, together with the unknown extent of the inaccuracies that are present, is there any point in analyzing data concerning perinatal mortality? In effect, one can only satisfactorily analyze perinatal data if there is a clear idea of what is being measured, if the comparison is of like with like, and if the conclusions take account of the limitations of the data.

It is essential to remember that statistics are a useful tool for describing patterns, but they can rarely be used to prove a causal hypothesis. The medical literature gives copious examples of unwarranted conclusions being reached from valid statistics. One of the examples that used to be quoted compared the annual number of newly registered mentally handicapped persons with the number of television sets bought. A positive correlation does not prove that watching television results in mental handicap.

Place of Birth

Closer to the subject of this book, Tew (1978, 1980, 1985, 1990) has published data that purport to show that delivery in a hospital results in more perinatal deaths than delivery at home, with the conclusion that home delivery is safest for the baby. Here the statistics are not wrong, and control for high-risk factors does not totally eliminate the association. The defect in her studies is that she is not comparing like with like.

To make the point clearer, suppose that instead of observational data she had been able to set up a randomized controlled trial in which n_1 mothers were allocated to home and n_2 to hospital delivery. Out of the n_1 randomized to deliver at home, x_1 will develop some complication that will necessitate their transfer to hospital care (e.g., rising blood pressure, placenta previa, proteinuria, abruptio placentae, suspected growth retardation, reduction in fetal movements, suspected fetal death, prolonged labor with failure to progress, etc.). Of the n_2 originally in the group for hospital care, x_2 will also develop these abnormalities.

There are now, at the point of delivery, $n_1 - x_1$ at home and $n_2 + x_1$ in the hospital. The only valid comparison is either between the $(n_1 - x_1)$ at home and the $(n_2 - x_2)$ in the hospital that did not develop complications, or between the original n_1 *intended* to deliver at home and the n_2 *intended* to deliver in hospital. When the latter strategy has been attempted on observational studies (Hobbs and Acheson 1966; Fedrick and Butler 1978), the data have shown that *intention* to deliver at home carries a higher mortality than *intention* to deliver in a consultant unit. This point has been discussed in more detail by Golding and Peters (1988).

Time Trends

Other examples where data are difficult to interpret are found in examining time trends. Many factors vary over time, from the social and geographical composition of the sample to features of obstetric practice. Therefore, it is not valid to observe, for example, an increasing use of fetal monitoring techniques and a falling perinatal mortality rate and claim that one has caused the other. A first step in an analysis in time trends would be to document the basic changes in the

population (including maternal age, parity, marital status, ethnic group, maternal smoking, prevalence of severe preeclampsia, birth weight, and gestation distributions). If a hospital population is being used, then it is vital to ascertain the numbers of mothers referred from outside the normal catchment area (these should be excluded) and those inside the area that have delivered elsewhere (these should be included).

Studies of Perinatal Mortality

Given that the biases inherent in case definition and ascertainment are known, there are four possible ways in which studies may be mounted. All have their advantages and disadvantages.

1. *National statistics*

a. *Advantages*: large numbers

b. *Disadvantages*: limited amount of information; cause-of-death data are largely meaningless (as described in the next section); possible under-ascertainment of deaths; lack of quality control on information (de Wals et al, 1989)

2. *National surveys*, using data specially collected over a defined period of time, e.g., Greek National Survey (Tzoumaka-Bakoula 1987); British National Surveys (Butler and Bonham 1963; Chamberlain et al. 1975); Jamaican National Survey (Ashley et al. 1994)

a. *Advantages*: improved quality of data; ability to classify deaths in a standard way; large amount of information available for analysis

b. *Disadvantages*: relatively small numbers of deaths unless the sample is extended, as in the First British Perinatal Mortality Survey (Butler and Bonham 1963) or the Jamaican study (Ashley et al. 1994)

3. *Ongoing area-based maternity information*

a. *Advantages*: quality control is possible, especially if organized so that research clerks abstract the information using well-formulated rules, with referral to consultant obstetricians in cases of difficulty (e.g., Cardiff Birth Survey and Aberdeen Survey)

b. *Disadvantages*: relatively small numbers of deaths in any one year; difficulty in accommodating within the system new obstetric and neonatal procedures introduced during the course of the study

4. *Hospital-based statistics*

a. *Advantages*: as in (3) above

b. *Disadvantages*: as in (3) above; in addition, the data are epidemiologically uninterpretable unless the hospital serves the whole of a geographical population; otherwise it is essential to ascertain also the outcome of pregnancy in all women resident in the referral area but delivered outside the hospital

5. *Prospective studies*

a. *Advantages*: surveys starting in pregnancy or even before have the benefit of greater accuracy in determining features relating to the mother and the pregnancy prior to the death occurring

b. *Disadvantages*: high cost; relatively small numbers

Causes of Perinatal Death

To identify ways in which perinatal deaths may be prevented, one needs a valid system of information and classification. Whether or not there has been a postmortem examination may not be considered important in developing countries with a lack of resources (Singh et al. 1988), but a number of studies in developed and developing countries have shown its importance clinically (Meier et al. 1986; Bétrémieux et al. 1989; D'Costa et al. 1995; Sutton et al. 1996) and for counseling the parents (Doyle 2000). It frequently will change the perception as to the cause or mechanism leading to death. A major resource implication concerns the advisability of a specialized pathologist able to identify the subtleties of diagnosis in perinatal autopsies (Rushton 1995).

Interpretation of the causes of death that appear on the perinatal death certificate is extremely difficult. Frequently, for example, the causes of death are entered in the medical chart by junior medical staff often before the results of the postmortem examination are known. Occasionally, the registered causes of death are given after necropsy, but even then a histological examination is unlikely to have been made, and the results of bacteriological and chromosome examinations or other tests are rarely available.

A number of studies have compared registered causes of stillbirth or neonatal death with postmortem findings (Fedrick and Butler 1972; Edouard 1982; Duley 1986), each showing poor correlation. Not surprisingly, overt congenital malformations are most likely to have been identified, but even for anencephalus, only 90% of cases had such a term on the death certificate (Fedrick and Butler 1972). At the other end of the spectrum, for a third of neonatal deaths ascribed to hyaline membrane disease there was no evidence at necropsy to support this (Duley 1986).

Much of the confusion in assigning causes to perinatal deaths lies in the multiplicity of contributing factors. For example, consider the following case: the mother, aged 17, was unmarried. She was smoking 30 cigarettes a day and had not received prenatal care until the third trimester of pregnancy, when she was found to have fulminating preeclampsia. Her membranes ruptured spontaneously and she went into labor at 35 weeks. A retroplacental hemorrhage was found, and the female infant was severely growth retarded. She was severely asphyxiated at birth but recovered rapidly after ventilation; nevertheless, after 4 hours she developed signs of respiratory distress and was treated appropriately but her condition deteriorated and she died 2 hours later. Necropsy showed resolving hyaline-membrane disease, a small ventricular septal defect, and an intraventricular hemorrhage.

Among the factors that might have been recorded on the death certificate are the following: maternal preeclampsia, maternal abruptio placentae, premature rupture of membranes, unmarried mother, heavy maternal smoking, preterm delivery, growth retardation, low birth weight, placental insufficiency, birth asphyxia, respiratory distress syndrome, intraventricular hemorrhage, and congenital heart disease. The International Classification of Diseases (ICD) would have assigned each of these factors a different code. One suggestion on methods of coping with multiple causes of death is that up to five different causes should be coded for each death. Even so, it is difficult to know which of the terms listed above would be most relevant; there are few published data to suggest what the underlying cause of death should be. The answer is clearly that we do not yet know. Certainly, the *risk* of perinatal death is greater if the mother is unmarried, a teenager, or a smoker, or if she develops preeclampsia, but that does not mean that in this particular case any of these factors contributed at all to this death. Unlike deaths in adults, where there is often an identifiable disease process and hence an underlying cause of death that would be recognized as such by many workers, in the perinatal field this rarely occurs. Winbo and colleagues (1998b) have emphasized the need for death certificates to be augmented with supplementary data in order to provide meaningful categorization, but even so a definitive cause or causes is unlikely to be identified.

Many authors have developed their own systems of classification, based on a combination of clinical and pathological findings (Baird et al. 1954; Hovatta et al. 1983), the pathological lesions found at necropsy (Claireaux 1962; Autio-Harmainen et al. 1983), placental and fetal pathology (Naeye 1977), or clinical features of the perinate (Wigglesworth 1980; Langhoff-Roos et al. 1996, 1998; Gardosi et al. 2005) or combinations of ICD codes from death certificates (Alberman et al. 1994; Winbo et al. 1998a).

Classification Systems

The following classification systems have been used more than once.

Aberdeen Clinicopathological Classification

The Aberdeen clinicopathological classification was first described by Baird and colleagues in 1954 and has been used extensively since then (McIlwaine et al. 1979). In the earlier paper the authors stated that necropsy findings usually indicate only the immediate cause of death and have little relevance from the point of view of etiology. As an example they cited an infant weighing 1.36 kg (3 lb) delivered by cesarean section, after placenta previa had been identified. Postmortem examination showed a tentorial tear, but the authors considered that placenta previa was a more meaningful cause of death than either prematurity or birth trauma. They developed very detailed rules for classification of perinatal deaths, almost entirely based on clinical information, and defined eight categories:

1. *Premature, cause unknown*:
a. Baby weighs <2500 g but is otherwise normal apart from possible maceration. Pregnancy and labor are normal even though onset of labor may have been preterm.
b. If lesions (such as intracranial hemorrhage or infection) are found at necropsy but baby weighs ≤1800 g and there have been no serious abnormalities of pregnancy.

2. *Mature, cause unknown*: pregnancy clinically normal, labor normal, baby weighing >2500 g.

3. *Mechanical*: all deaths without major malformations, with baby weighing >1800 g, with breech or shoulder presentation, or with obstruction of the cord. In vertex deliveries, the degree of molding and characteristics of labor are taken into account. Diagnosis of trauma may be made in the absence of necropsy evidence of birth injury.

4. *Toxemia*: intrauterine or neonatal death caused by prematurity or anoxia when eclampsia or severe or moderate preeclampsia have been present. When antepartum hemorrhage has also occurred, cases are classified as toxemia.

5. *Antepartum hemorrhage*: all cases with placenta previa, accidental hemorrhage, or antepartum hemorrhage of uncertain origin.

6. *Maternal disease*: "cases in which the mother has suffered during pregnancy from an incidental medical or surgical condition such as diabetes, pneumonia, syphilis, or appendicitis, apparently leading to intrauterine death or to the expulsion of a feeble, toxic (usually premature) baby which dies in the first week."

7. *Deformity*: malformations incompatible with life or likely to have been the prime factor contributing to death.

8. *Other causes*: includes cases in which mother had rhesus antibodies and cases in which infants, healthy at birth, died of infection, hemorrhagic disease, etc. Subsequently this class was divided into two groups: blood group incompatibility, which took precedence over all other causes except deformity, and the remainder.

This classification has been used in a number of studies of perinatal death; thus it is interesting to compare the results (Table 9.1). As expected, the proportion of deaths "caused by" trauma and blood group incompatibility has fallen dramatically, whereas the proportion associated with malformation and prematurity (cause unknown) has risen.

Within the last few years there have been two attempts to update and rationalize the classification. On the one hand Cole and colleagues (1986) suggested a number of subdivisions within each group, but Whitfield and colleagues (1986) recommended more substantial changes to identify 12 subgroups, as shown in Table 9.2.

For a classification system to be useful it should be reproducible. A study of 451 perinatal deaths compared the classifications given by pediatricians, obstetricians, general practitioners, and midwives. The agreement (kappa = 0.55 for the

Table 9.1. Distribution of perinatal deaths according to the Aberdeen Clinicopathological Classification

Clinical classification	Aberdeen*1938–1952 (%)	Great Britain† 1958 (%)	Scotland‡ 1977 (%)	Northern Region§ 1981–1982 (%)
Premature, cause unknown	19.7	17.4	29.8	31.9
Mature, cause unknown	13.7	15.1	10.5	12.4
Trauma	18.8	13.1	4.9	2.7
Toxaemia	10.0	13.1	8.8	7.1
Antepartum hemorrhage	10.9	13.1	12.5	15.0
Malformation	15.6	18.0	26.2	21.2
Maternal disease	6.0	2.4	4.4	4.4
Other		2.4	1.3	3.5
Incompatibility	5.3	4.8	1.6	0.9
Number of deaths (=100%)	1008	2210	1012	988

*Cases booked at Aberdeen Maternity Hospital (Baird et al. 1954).
†First National Perinatal Mortality Survey (Baird and Thomson 1969).
‡The Scottish Perinatal Mortality Survey (McIlwaine et al. 1979).
§The Northern Regional Health Authority Coordinating Group (1984).

Table 9.2. Modification of the Aberdeen classification of perinatal deaths

Aberdeen classification*	Whitfield classification†
1. Premature unknown	• Spontaneous preterm • Intrauterine growth retardation • Unexplained IUD
2. Mature unknown	• Intrapartum asphyxia
3. Mechanical	• Trauma
4. Toxemia	• Hypertension
5. Maternal disease	• Maternal disease
6. Antepartum hemorrhage	• Antepartum hemorrhage
7. Deformity	• Fetal abnormality
8a. Blood group incompatibility	• Hemolytic disease
8b. Other	• Infection • Other

*Baird et al. (1954).
†Whitfield et al. (1986).

obstetric classification; Cole et al. 1986) was poor (Settatree and Watkinson 1993).

British Necropsy Classification

For the 1958 British Perinatal Mortality Survey (BPMS), Claireaux (1962) developed a classification based on postmortem findings. His results are compared in Table 9.3 with those from the perinatal mortality survey in Cuba (Rojas Ochoa 1981) and from Machin's (1975) study in London. As in other systems, hierarchical decisions had to be made. For example, evidence of both intrauterine asphyxia and cerebral birth trauma was always classified as trauma; an infant with pneumonia and another major lesion was always grouped with the other lesion.

Table 9.3. Distribution of deaths according to British necropsy classification

Classification of primary anatomical lesion	BPS* 1958 (%)	London† 1970–1973 (%)	Cuba‡ 1973 (%)
Congenital malformation	15.1	23.8	12.3
Blood group incompatibility	4.0	3.3	2.6
Antepartum asphyxia	13.4	21.3	14.9
Intrapartum asphyxia	20.8	20.9	22.7
Intracranial birth trauma	8.9	4.0	4.2
Intraventricular hemorrhage	2.4	10.6	3.7
Hyaline membranes (HMs)	5.8	6.2	11.4
Massive pulmonary hemorrhage	2.7	1.2	2.1
Pneumonia	4.1	5.0	6.9
Extrapulmonary infection	0.3	1.2	2.9
Other lesion	1.1	4.9	1.9
Resorption atelectasis (no HMs)	3.3		
No anatomical lesion found	18.1	0.6	14.2
Number of deaths (=100%)	2368	721	1400

*Claireaux (1962) (population-based).
†Machin (1975) (hospital-based).
‡Rojas Ochoa (1981) (population-based).

That there were disadvantages in assuming that one lesion was the most important rapidly became apparent both to the team analyzing the BPMS data and to Valdes-Dapena and Arey (1970) in the United States. Later analyses of the BPMS deaths examined all deaths with a particular pathological abnormality and looked at the interactions with other lesions, as well as with clinical factors (Fedrick and Butler 1970a,b, 1971a–d).

A different attempt to address the problems inherent in multiple causes has been attempted by Hey and colleagues (1986). They suggest dividing the congenital malformations category into six groups (chromosomal, inborn error of metabolism, neural tube defect, congenital heart disease, renal abnormality, and other malformation), the hyaline membrane disease (HMD) group into three classes [HMD, HMD + intraventricular hemorrhage (IVH), HMD + infection], and the two infection groups into four (necrotizing enterocolitis, antepartum infection, intrapartum infection, and postpartum infection). They suggest there should be further categories for "other intracranial bleeding," "cot death," "unattended delivery," and "undocumented or unclassified." Comparability between observers (Settatree and Watkinson 1993) showed relatively poor consistency with this classification (kappa = 0.58).

Placental and Fetal Pathology Classification

Naeye (1977) classified causes of perinatal death according to placental and fetal pathology as follows:

1. *Acute amniotic fluid infection syndrome*: acute congenital pneumonia associated with acute inflammation of extraplacental membranes, acute funisitis, acute inflammation of the chorionic plate of the placenta. Membranes had to have been intact at start of labor.
2. *Abruptio placentae*: clinical evidence of abruption and retroplacental clot and evidence of

fetal hypoxia, e.g., aspirated squamae and petechiae on surface of visceral organs.

3. *Premature rupture of membranes*: if <37 weeks' gestation: membranes rupture prior to onset of labor; if ≥37 weeks, membranes rupture at least 20 hours prior to onset of labor.

4. *Congenital anomalies*: severe and incompatible with survival.

5. *Large placental infarcts*: at least 25% of placenta involved and by one or more infarcts over 3 cm in diameter; no other known explanation for death.

6. *Intervillous thrombi of placenta*: if more than 3 cm in diameter and involving at least 25% of placenta.

7. *Umbilical cord compression*: breech delivery with cord compressed by head, or cord prolapse, or cord tightly round neck.

8. *Cord knots*: if knots were tight, no other explanation for death.

9. *Placental growth retardation*: placenta 40% below weight for gestation and no other explanation for death.

10. *Placenta previa*: placenta encroached on the cervical os and death caused by blood loss or consequences of premature delivery.

11. *Rhesus erythroblastosis.*

12. *Birth trauma*: not defined by Naeye, but 91% of his group of cases had subdural hematomas.

13. *Polyhydramnios*: excess amniotic fluid initiated preterm labor and infant died from consequences of immaturity.

14. *Cesarean section*: gestational age overestimated and infant died from consequences of immaturity.

15. *Marginal sinus rupture*: a marginal tear in the placenta, which appeared responsible for antepartum hemorrhage and fetal hypoxia.

16. *Severe fetal undernutrition*: gross and microscopic characteristics (undefined) of undernutrition without any known causes except maternal weight loss or poor weight gain.

17. *Uterine rupture.*

18. *Postmaturity*: gestation ≥44 weeks, gross and microscopic evidence of undernutrition and no other explanation for death.

19. *Congenital syphilis*: spirochetes in the infant's tissues.

20. *Other disorders.*

Table 9.4. Mortality rates (per 1000) attributable to placental/fetal pathology

Naeye's classification	Durban, South Africa	Addis Ababa, Ethiopia	U.S.
Amniotic fluid infection	14.2	17.5	5.9
Abruptio placentae	8.1	4.5	3.8
Premature rupture of membranes	1.1	1.1	3.5
Congenital anomalies	2.7	2.4	3.2
Large placental infarcts	0.9	1.5	2.1
Umbilical cord compression	2.2	3.4	1.2
Placental growth retardation	0.5	0.2	0.9
Placenta praevia	1.0	1.6	0.7
Birth trauma	3.9	0.9	0.5
Congenital syphilis	3.2	4.3	0.1
Fetal hypoxia	6.5	3.6	2.6
Obstructed labour	3.3	8.4	—
Other acute infections	0.9	0.3	0.4
Other disorders	4.8	3.5	3.9
Diagnosis unknown	0.8	0.2	4.6
Total	54.6	53.4	33.4

Data abstracted from Ross et al. 1982.

The rates in Table 9.4 indicate the proportion of stillbirths and neonatal deaths that were placed in each category using deaths from the U.S., South Africa, and Ethiopia. It will be noted that categories 6, 8, 11, and 13 to 18 in the above list have been replaced by fetal hypoxia, obstructed labor, other acute infections, or amalgamated into the "other" category.

Wigglesworth Classification

The classification proposed by Wigglesworth (1980) is an attempt to produce a simple system for classifying perinatal deaths and purports to be relatively accurate whether or not postmortem examination is carried out. There are five categories:

1. Normally formed antepartum fetal death
2. Congenital malformation
3. Conditions associated with immaturity
4. Asphyxial conditions developing in labor
5. Specific conditions other than the above

The five categories seem to be fairly straightforward, but problems arise in a minority of cases. For example, should one really put a macerated stillbirth with a minor isolated defect (e.g., polydactyly, cleft palate) in category 2, rather than category 1? How does one classify the neonatal

death of an infant of weight 3500 g and 41 weeks' gestation who died with an intraventricular hemorrhage? The cause of death is certainly one that is usually associated with immaturity even though the index infant was mature.

Barson and colleagues (1984) attempted to address such ambiguities. They suggested that a malformation that could be lethal if found in isolation took precedence over all other causes and that preterm infants should be placed in category 4 if there was profound hypoxia at birth unresponsive to resuscitation. They included neonatal necrotizing enterocolitis and meningitis in category 5 but placed preterm infants with pneumonia in category 3. Nevertheless, these modifications still leave a number of unanswered questions, which were addressed by Keeling and colleagues (1989), who set out clearer rules in order to clarify ambiguities.

Comparison of various British studies using the Wigglesworth classification is shown in Table 9.5. It can be seen that the proportion of normally formed macerated stillbirths has increased, but those associated with intrapartum asphyxia and congenital malformations have decreased. There is increasing evidence of the validity of using this classification. An important study (Lumley and Bakoula 1993) has compared perinatal mortality in Greece with that of Greeks living in Australia. They showed that the reduced perinatal mortality rate in Australia was entirely due to a reduction in the intrapartum asphyxia group.

The simplicity of the classification was demonstrated when Winbo and colleagues (1997) showed that a computer algorithm to classify deaths from Swedish medical registries showed 88% concordance when compared with an independent classification of the medical records. Comparison of the classification given by four different observers showed good reliability (kappa = 0.67) (Settatree and Watkinson 1993). The classification has also been found to be particularly useful in developing countries. In Jamaica the major category was intrapartum asphyxia, accounting for 44% of perinatal deaths. Analysis of the data showed that the mortality rate associated with intrapartum asphyxia was reduced in areas with access to specialist obstetric and neonatal facilities (McCaw-Binns et al. 1994a).

There has been a variety of classifications based on Wigglesworth but using an "extended" categorization; these classifications have proved less reliable than the original. Thornton and O'Hara (1998) found that autopsy findings resulted in changes in classification in 21% of deaths, whereas the original grouping was changed in only 9% of perinatal deaths when classified before or after consideration of postmortem findings (Keeling et al. 1989).

Other Classifications

An international collaborative effort was made in the 1980s to use information on neonatal death certificates to form a classification (Cole et al. 1989). Detailed assessment showed that this effort provided very inaccurate categorization when compared with the Wigglesworth classification (Winbo et al. 1998b).

A Nordic-Baltic classification was used in the 1990s to compare types of perinatal death between Denmark and Sweden (Langhoff-Roos et al. 1996)

Table 9.5. Comparison of British studies using the Wigglesworth classification

Wigglesworth	BPMS*1958 (%)	Manchester† 1976–1981 (%)	Northern Region‡ 1988–1995 (%)	SE Thames Region§ 1981–1982 (%)
Antepartum fetal death	21	29	41	41
Malformation	19	25	22	14
Immaturity	12	24	15	26
Intrapartum asphyxia	32	16	17	9
Other	16	6	5	10
Number of deaths (= 100%)	2368	440	988	3716

*Adapted from Butler and Bonham 1963.
†Barson et al. (1984).
‡Northern Regional Health Authority Coordinating Group (1984).
§Hanson and Reynolds (1998).

and with Lithuania (Langhoff-Roos et al. 1998). The classification is based mainly on time of death and gestation and comprises 13 different classes. Comparison of Danish and Swedish data had shown excess deaths related to congenital malformations and intrapartum factors (Langhoff-Roos et al. 1996), and comparison of these data with those derived for Lithuania showed no significant difference in rates of antepartum fetal death, but excess deaths in all other categories.

More recently, a categorization of Neonatal and Intrauterine Death Classification according to Etiology (NICE) has been developed (Winbo et al. 1998a). The 11 categories bear some relation to the Aberdeen classification. NICE has been used to compare causes of death in different areas of Sweden (Serenius et al. 2001). Yet another categorization of stillbirths has been published (Gardosi et al. 2005) that is based on the claim that in most classifications there is a large proportion of unexplained deaths. The new proposal ReCoDe (relevant condition at death) has 37 categories. It was used on 2625 stillbirths, and only 15% were unclassified; however, as many as a further 43% were put in the category "fetal growth restriction," which as pointed out subsequently (Kirk 2005; Sebire 2005) is arguably a different way of stating that the actual cause of death was unknown.

Avoidability of Death

Given so many different classifications of perinatal death, and so many factors that influence the risk of death, the staff members assessing each death are likely to bring their own experience, expertise, and biases into play. To address this problem, there has been a move to institute confidential inquiries using a committee approach (Chalmers 1985). This requires a subjective decision to be made about the possible avoidability of death.

The etiology of perinatal deaths is extraordinarily complex. I agree with Baird (1980), who stated, "I think it is a mistake to talk about avoidability of perinatal deaths. Who can tell whether a perinatal death could have been avoided? What one can do is describe the circumstances in which the death occurred and the type of death."

Nevertheless, a number of regions have undertaken such studies. If the criterion of "avoidable death" was meaningful, and local efforts were made to remedy any faults in obstetric or neonatal practice that might have been identified, then it is reasonable to postulate that subsequent mortality might be reduced. One study (Thomas et al. 1985) claims that it has, but Elbourne and Mutch (1984) compared the improvements in mortality rates in all those British regions that had ongoing confidential inquiries with the areas that had no such monitoring system. The fall in mortality was similar in each group, thus suggesting that, although of interest locally, it was unlikely that such inquiries would actually prevent many deaths.

Confidential inquiries at the national level have identified many episodes of suboptimal care when perinatal death has occurred [Confidential Enquiry into Stillbirths and Deaths in Infancy (CESDI) 1997]. Detailed records and reports are needed for realistic interpretation of shortfalls in care. Consistency of peer review is difficult to achieve in large studies, and definition of standards and a structural reviewing procedure are important in this respect (CESDI 1998). These studies do, however, permit identification and in-depth study of rare events producing guidelines for management and highlighting the need for problem-specific training programs (CESDI 1998).

Cause or Association

Baird and colleagues (1954) produced a logical argument to support the largely clinical classification that they advocated (the Aberdeen Clinicopathological Classification), as opposed to a classification based on necropsy findings, on the basis that the clinical abnormalities usually resulted in the lesions that led to the death of the fetus. Yet, over 40 years on from their original publication are we actually able to say that the death of an infant born to a mother with moderate preeclampsia is the result of that maternal disease? Perusal of the statistics shows that perinatal deaths are 30% and 130% more likely if the mother has either moderate or severe preeclampsia. This implies that out of every 130 deaths to mothers

with moderate preeclampsia, 100 would have been expected to occur anyway, and thus only 30 would actually be associated with the preeclampsia. Are we yet in a position to assert positively that we are able, either from clinical history or postmortem examination, to distinguish the 100 from the 30? Until we can do this it would surely be wrong to use only the clinical classification.

What then should be used? The answer must surely depend on the use to which the system is put. Not unexpectedly, obstetricians have found the Aberdeen clinical classification to be the most useful, and only pathologists appear to have used the mainly anatomical classifications (Machin 1975; Naeye 1979a). Consequently, epidemiological analyses of factors associated with specific causes of death are difficult to compare with one another.

The need for a simple system that is useful to those who are interested in interpreting perinatal death data may be answered by the Wigglesworth classification. This has been shown to have validity and repeatability regardless of the experience of the classifier and the extent of postmortem examination (Keeling et al. 1989; Settatree and Watkinson, 1993). It is increasingly being found to be of great value in monitoring trends in perinatal mortality and in comparing different populations, in both developed and developing countries (Amar et al. 1996). The Nordic-Baltic classification is based on a more complex categorization and is slightly less easy to interpret. Nevertheless, it could be very useful. If neither of these is feasible, the best option may be to follow the guidelines of the International Federation of Gynecology and Obstetrics (FIGO) Standing Committee on Perinatal Mortality and Morbidity (1982) and compare perinatal deaths of birth weight ≥1000 g, both including and excluding lethal malformations. This procedure has proved helpful in local studies (Mutch et al. 1981), but nevertheless still requires complete ascertainment of perinatal deaths.

Maternal and Environmental Factors Associated with Perinatal Death

When studies have been carried out on populations, a number of consistent and striking associations have been found for perinatal mortality in general. These are described in the following subsections.

Maternal Age

There is generally a U-shaped variation with the risk of perinatal death, very young and older mothers having the highest rates (Cahalane et al. 1965; Naeye and Tafari 1983; Pharoah and Alberman 1988). However, there are differences in pattern according to time of death, with the stillbirth rate increasing with maternal age and a shallow U-shape for neonatal deaths (Golding 1990a). In Jamaica an attempt was made to analyze the specific mechanisms involved. The authors found that the relationship between advanced maternal age and stillbirths was mainly associated with antepartum fetal deaths, and was explained by the increased rates of hypertension, bleeding, and syphilis in older mothers (Greenwood et al. 1994a). The same survey demonstrated a marked increase in risk of Wigglesworth group 3—neonatal deaths associated with immaturity—among the births to teenagers, that could not be explained by specific complications of pregnancy (McCaw-Binns et al. 1994b).

Parity

Parity is measured as the number of previous pregnancies resulting in live- or stillbirth. The parities with least risk of perinatal death are parities 1 and 2 (i.e., second and third pregnancies). Thereafter, in general, the risk rises with increasing parity. There is some evidence that the difference in risk between parities 0 and 1 has decreased over time (Golding 1990a), but it is still apparent in most countries (Golding 1990b).

Past Obstetric History

History tends to repeat itself. Sometimes the repetition is the result of similar combinations of genes in the conceptuses, but more often it is a continuing biological abnormality or environmental hazard in the mother. Whatever the reason, the most important factor in predicting the risk to the current pregnancy is the outcome of previous pregnancies of the same mother. In Britain, a history of previous miscarriages

resulted in a 68% increase in perinatal death, but the increase was 200% if there had been a previous perinatal death (Fedrick and Adelstein 1977). In Jamaica, the risk was not significantly increased when there had been one miscarriage but was increased by 76% when there had been two or more previous miscarriages, by 128% in the presence of previous stillbirth and by 124% if there had been a prior early neonatal death. The risk to the current pregnancy was always increased further if the previous loss had occurred in the immediately preceding pregnancy (Greenwood et al. 1994b).

Interpregnancy Interval

There is much confusion in analysis of interpregnancy interval. It is essential that this be measured as the length of time from the preceding delivery to the last menstrual period (LMP) of the index pregnancy. This becomes problematic in mothers with unknown LMP, or who conceived again prior to menstruating; however, these problems can be overcome with clinical or necropsy estimation of gestation. Women with preceding miscarriage or perinatal loss tend to have very short interpregnancy intervals. Thus, analysis must always take account of the past obstetric history. There is now reliable evidence from Britain and Norway that a prolonged interval between pregnancies carries an increased risk of stillbirth, but that a short interval (i.e., less than 6 months from preceding delivery to current conception) carries an increased risk of neonatal death (Fedrick and Adelstein 1973; Bakketeig et al. 1984).

Ethnic Group

In the U.S., perinatal mortality rates among the black population are greater than among whites; in Australia, the aborigines have a higher mortality rate than the white settlers; in Israel, the Arabs have a higher mortality than the Jews; in Sweden and the U.K., immigrants have a higher perinatal mortality than the population of parents born in the country. Reasons for the differences are multiple and vary from country to country. In the U.K., immigrants from India, Bangladesh, and Pakistan have the highest rates (Gillies et al. 1984), much of the excess being associated with congenital malformations. Some of the differences may be due to inbreeding (consanguinity rates in these groups are very high), to dietary differences, especially the vitamin D deficiency that develops following arrival in the U.K., or to socioeconomic factors.

Social Class

In the U.K., the classification of social disadvantage that most clearly reflects differing risks in perinatal mortality is based on a classification of the father's occupation, into the following classes: I, higher professionals such as doctors, ministers of religion, barristers; II, other professionals and managers, including teachers, nurses, company directors; IIIN, skilled nonmanual, such as salesmen, clerks, draftsmen; IIIM, skilled manual, such as plumbers, carpenters, truck drivers; IV, semiskilled, such as caregivers, machinists, letter carriers; V, unskilled, including cleaners and laborers. Single mothers are not included in this classification. In general, the perinatal mortality rate in social class V is almost twice that in social class I (Pharaoh and Alberman 1985).

Other classifications have used parental education and have shown that there is often a strong trend, with the highest perinatal mortality rate in parents who had the lowest level of education (Golding 1990a). Nevertheless, in Greece a U-shaped variation has been found where women with lowest and highest education levels had the highest mortality rates (Tzoumaka-Bakoula et al. 1989), but in Jamaica there is no independent effect of maternal education level (Golding et al. 1994).

Single Parents

In countries where, and in periods when, the unmarried state was relatively unusual, there was a marked increase in mortality rates to single mothers (Golding 1990a), but in countries where unmarried deliveries are not unusual, it is still accompanied by an excess of perinatal deaths (Golding 1990c; Skjaerven and Irgens 1988). It has been shown that there is an increase of about 40% in the risk to the fetus of an unmarried mother in

Britain, over and above that related to her age and parity (Golding et al. 1986). In Jamaica, there is evidence that unmarried mothers living in a stable relationship with the father of the child are also at increased risk, but mothers who are living apart from the father of the child are at much greater risk (Golding et al. 1994).

Maternal Size

Prepregnant maternal weight has only occasionally been studied in relation to perinatal mortality. In the U.S., the risk increased with increasing weight (Naeye 1979b), but in Brazil a relationship was found for stillbirths but not for neonatal deaths (Barros et al. 1987). In general, both studies have shown an increase in risk with low maternal weight gain, but this finding is uninterpretable, being confounded by the fact that women who deliver preterm are bound not to have put on as much weight during pregnancy as women going to term. In the U.S., mothers who put on excessive weight were at increased risk, but this was not true of mothers in Brazil.

Maternal height was considered important when the British Perinatal Mortality Survey showed an increased risk in short mothers (Butler and Bonham 1963), but this was not found in the U.S. (Naeye and Tafari 1983). In Brazil there was no relationship with stillbirth, but for neonatal deaths there was increasing risk with decreasing maternal height (Barros et al. 1987). In Jamaica the group of mothers with average heights had the highest risk and both shorter and taller mothers had significantly lower risks (Greenwood et al. 1994c).

Maternal Smoking

There is strong evidence that perinatal mortality risk increases with number of cigarettes smoked, and that this association is particularly strong for stillbirths (Cnattingius et al. 1988; Kleinman et al. 1988; Raymond et al. 1994; Tuthill et al. 1999). Among stillbirths the association appears to be with antepartum rather than intrapartum fetal deaths (Tuthill et al. 1999). Further studies have indicated that the risk is much reduced if mothers have ceased to smoke by 16 weeks' gestation (Wisborg et al. 2001).

Alcohol Consumption

Although possibly implicated in spontaneous abortion (Kline et al. 1980), there is no clear evidence that mild or moderate alcohol consumption has an adverse effect on the fetus. Indeed, in Jamaica mothers who drank some alcohol had a slightly reduced risk of losing their baby (Greenwood et al. 1994c). In contrast, the fetus of the alcoholic mother is certainly at risk of growth retardation and dysmorphism, but it is not clear whether perinatal mortality rates are elevated.

Diet

Although it is often assumed that maternal diet has a major impact on perinatal mortality, the evidence is lacking. Supplement studies are rarely large enough to show any effect on perinatal mortality, and studies that have looked at general aspects of the diet have very rarely taken account of other features that may be contributing to perinatal mortality (Golding 1990a). Of the supplement studies that have been carried out, a calorie protein supplement compared with placebo resulted in a reduction in perinatal mortality in Germany, which was just statistically significant (Mora et al. 1978). In the U.S., a randomized controlled trial of high-protein supplements resulted in a higher neonatal death rate when compared with controls receiving a placebo (Rush 1986). In Jamaica, women taking iron supplements were significantly less likely to have a perinatal death (Greenwood et al 1994c); in addition, those taking folate were less likely to have a death from immaturity (McCaw-Binns et al. 1994b). In general, however, it is now considered that there may be a reduction in fetal death of the order of 2 per 1000 with appropriate dietary intervention but only in populations with poor diet in general (Rush et al. 1988).

Preeclampsia

Although severe maternal preeclampsia is associated with a greatly increased risk of mortality in the infant, mild preeclampsia (defined as a rise to maximum diastolic of 90 to 99 mm Hg in the third trimester) carries no increase in risk at all (Butler and Bonham 1963). It is therefore essential to

determine the degree of preeclampsia before ascribing it as a contributory cause of death.

Bleeding

A history of bleeding in pregnancy is associated with elevated risk of perinatal death. Most attention is paid to pregnancies with placenta previa or where accidental hemorrhage has developed. Certainly, both have high associated mortality rates (Record and McKeown l956; Naeye et al. 1977; Naeye 1978; Huisjes et al. 1979). Nevertheless, numerically more deaths are associated with unspecified antepartum hemorrhage (Butler and Bonham 1963), or even bleeding in the first two trimesters, which has been shown to predict a threefold increase in perinatal mortality (Ananth and Savitz 1994).

Other Maternal Disorders

Data from the U.S. Collaborative Study have indicated that perinatal mortality rates are elevated in the presence of maternal heart disease, asthma, epilepsy, urinary infection within 15 days of delivery, glomerulonephritis, and diabetes (Naeye and Tafari 1983). From the major Jamaican study there was no relationship with urinary infection but marked associations with maternal syphilis, diabetes, and vaginal infection (Greenwood et al. 1994c).

Other Factors

There is no firm evidence that poor housing conditions or lack of employment per se result in higher mortality rates in the developed world. In the developing world, however, there are reported associations with type of toilet (Greenwood et al. 1994c) and crowding within the household (Rahman et al. 1985).

There is some prospective evidence that stressful life events in pregnancy may result in the onset of preterm labor (Newton et al. 1979) and hence an increased risk of neonatal death. There is also prospective evidence that if the mother had, at the start of pregnancy, a negative attitude to pregnancy there is an increased risk of perinatal death (Laukaran and van der Berg 1980).

Characteristics of the Infant

Sex of Fetus

Stillbirth rates do not vary markedly with the sex of the fetus, but neonatal death rates are higher in boys, largely due to the fact that boys are more likely than girls to be delivered prematurely (Karlberg et al. 1990).

Birth Weight

In all countries a distinctive pattern is found when perinatal mortality rates are plotted against birth weight. There is a marked reduction in risk with increasing birth weight up to 3500 g, and then an upturn, with heavier infants being slightly more likely to die.

Low birth weight, however, is made up of three major components: multiple births, growth restriction, and preterm delivery.

Multiple Births

The perinatal mortality rate in twins is at least four times that of singletons (Golding 1990d; Imaizumi 1994). In addition, the second twin is at 35% greater risk of neonatal death than the first twin. Mortality of twins weighing under 1500g is similar to that of singletons of the same weight; mortality of twins weighing 1500 to 2500 g is less than that of singletons of the same weight; mortality of twins weighing 2500 g or more is higher than that of singletons of similar weight (Golding 1990d). Mortality of triplets is some 12 times and quadruplets 15 times that of singletons (Imaizumi 1994).

Growth Restriction

Whether measured in terms of percentiles or standard deviations from the mean, infants at the mean or median have the lowest risk of perinatal death and those at the lowest extreme (i.e., with marked growth restriction) have the highest risk.

Preterm Delivery

The gestation with the lowest perinatal mortality rate is in the period 39 to 41 weeks. From there, the lower the gestation the higher the risk, but

there is also an increased risk among the postmature (Hilder et al. 1998).

Preterm Delivery and Growth Restriction

It is important to remember that, in spite of the patterns above, for a given birth weight the growth-retarded fetus has a lower mortality than the normally grown, but that the growth-accelerated fetus has a higher perinatal mortality rate than normally grown fetuses of the same weight.

Genetics

Although there is a recurrence effect, in that mothers who have had one perinatal death are at increased risk of further perinatal deaths, this does not necessarily imply a genetic effect. It could equally apply to continuing environmental conditions or medical problems in the mother. Nevertheless, one particular cause of perinatal deaths was certainly associated with a specific gene in the past. This occurred when rhesus-negative mothers were exposed to rhesus-positive fathers in one pregnancy and then reacted against any subsequent pregnancy that was rhesus positive. This cause of perinatal mortality has fallen rapidly in association with primary preventive methods involving the administration of anti-D after delivery (Clarke et al. 1985).

As to whether consanguineous mating results in increased risk of perinatal mortality over and above that associated with congenital malformations, there has been conflicting evidence. The best data are probably those collected by Stevenson and colleagues (1966). Their international study found a relative risk of perinatal death among consanguineous matings of 1.73 in comparison with unrelated parents. Nevertheless, no analysis was done to assess whether this was an artifact; it is possible that consanguineous mating may be more prevalent in the lower social groups or in religious groups that have particular habits that are harmful to the fetus. More recently, logistic regression has been used to demonstrate a twofold increase in risk of perinatal mortality when the parents were first cousins (Hussain 1998).

Conclusion

This chapter has shown how perinatal mortality rates vary with the different definitions used, and that registration of perinatal deaths is rarely if ever strictly accurate. The cause of death classification varies from center to center, but in general the Wigglesworth classification appears to be simpler and more repeatable than many others. It has the added advantage of being interpretable by both clinicians and lay personnel.

The factors contributing to perinatal mortality are varied, and point mostly to environmental factors. Nevertheless, the mechanism of these relationships has rarely been identified. Further progress into the understanding of perinatal deaths should involve studies of geographically defined populations with accurate data collection on both deaths and the background control population. The epidemiological method can assess ways in which perinatal mortality rates are higher than expected, and can give pointers to appropriate preventive strategies.

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10
Macerated Stillbirth

Isabella E. Moore

Despite the increasing accuracy of prenatal detection of birth defects and genetic disorders, necropsy still provides the final diagnosis in many cases and serves as an "ultimate audit." In Berger's (1978) view, "any single autopsy may not have dramatic import for progress in medicine, but it may have profound and lifelong implications for helping the dead child's family." Necropsy results are of great importance to the parents of stillborn infants.

In recent years, a fall in intrapartum deaths has been observed as part of the decline in overall perinatal mortality rates in developed countries [Georgsdottir et al. 1989; Confidential Enquiry into Stillbirths and Deaths in Infancy (CESDI) 1997]. Thus a larger proportion of perinatal deaths is now attributable to antepartum causes. Antepartum stillbirths may comprise more than one third of perinatal deaths (Keeling 1987). To achieve even lower perinatal mortality rates, efforts should be focused primarily on the investigation of antepartum deaths.

The importance of adequate pathological examination of both fetus and placenta has been emphasized in several publications (Rushton 1991; Cartlidge 1995). The examination of a macerated fetus should not be looked upon as a low-priority exercise. The necropsy of a macerated baby may not provide an exact cause of death but it can produce valuable information concerning the time of death and details of fetal growth and development, and identify unsuspected malformations. All this information assists the obstetrician in counseling the distressed parents who experience the death of their child without any warning during the course of an apparently normal pregnancy.

Even in the absence of parental consent for postmortem examination, weight and external measurements, whole-body x-ray, photographs, placental examination, and, exceptionally, ultrasound scan or magnetic resonance imaging can be performed (Brookes et al. 1996).

Changes Associated with Maceration

Maceration (Latin *macerare,* to soften by soaking) describes the softening effect of soaking on solid tissues and is applied to the degenerative changes occurring in a fetus retained in utero after death. Careful recording of the extent and appearance of cutaneous maceration can provide useful information about the time of death (Genest and Singer 1992). Autolysis, due to endogenous proteolytic enzymes, contributes to softening of the tissues and should not be confused with maceration. The earliest signs of maceration are seen in the skin in the form of skin slipping (Strachan 1922), approximately 6 hours and certainly 12 hours after intrauterine death (IUD). The epidermis can be easily separated from the dermis by applying oblique pressure (Fig. 10.1). Loss of the epidermis exposes a red, shiny, moist dermal surface. This is particularly noticeable over bony prominences. Approximately 24 hours after IUD, fluid-filled bullae are formed between dermis and epidermis. Fetal epidermis loses its integrity within the first 3 to 4 days and scalp edema can be seen on the ultrasound scan (halo sign). Up to 5 mm of

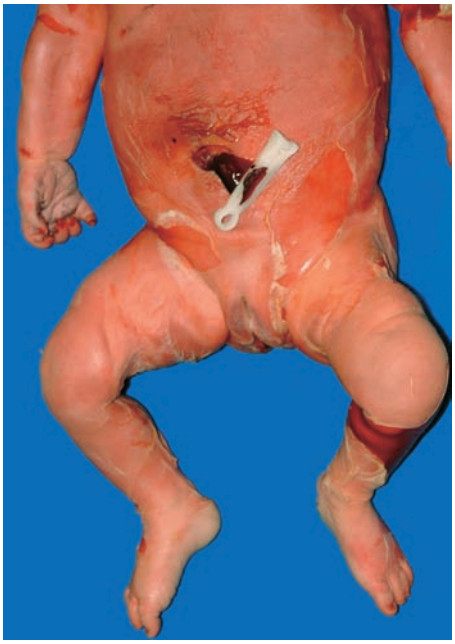


Figure 10.1. The earliest sign of maceration is skin slippage following obliquely applied pressure, revealing a shiny, moist dermal surface.

subcutaneous edema may be present by the fifth day after IUD (Hill et al. 1989). Long-standing IUD may occasionally mimic hydrops fetalis, and unusual sonographic appearances associated with IUD should be distinguished from true pathological abnormalities. For example, fluid accumulation at the nape of the neck related to advanced maceration should not be mistaken for a cystic hygroma or occipital encephalocele.

Approximately 48 hours after death, the internal fetal organs and connective tissue show increasing purple discoloration due to hemolysis and breakdown of red cells. Dark, red-stained fluid accumulates in the serous cavities. This should be distinguished from serous effusions acquired antemortem. Proteolytic digestion by kallikrein contributes to increased vascular permeability in a macerated fetus (Singer and MacPherson 1991).

Similar color changes are observed in the amniotic fluid, which has a deep dark red appearance ("tobacco juice"), or in the event of passage of meconium, a thick brown appearance. The volume of the amniotic fluid diminishes following fetal death, and the level of α-fetoprotein in it may be raised because of concentration effect and increased fetal skin permeability.

Autolysis of connective tissue contributes to laxity of joints and lack of definition of cut surface margins of the solid organs. The abdominal organs may show green discoloration due to leakage of bile pigments from the gallbladder. In an IUD occurring not less than a week previously, meconium may be released into the abdominal cavity through the dissolving bowel wall. Occasionally, the protruding autolyzed liver mass can produce an abnormal ultrasonographic appearance that could be mistaken for omphalocele (Hill et al. 1989) (Fig. 10.2). Dystrophic calcification may develop in the autolyzed liver tissue.

After 4 to 5 days, cranial vault bones become separated from the dura and periosteum. Overlapping of the bones produces the characteristic Spalding sign on ultrasound examination. Maceration may permit distortion of the skull during vaginal delivery and may lead to an erroneous diagnosis of hydrocephalus. Squeezing of the

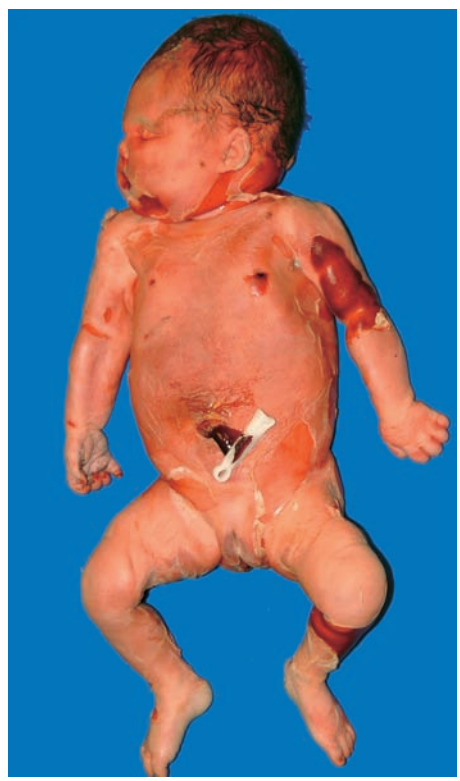


Figure 10.2. Severely macerated stillborn infant: the exposed dermis shows dark red discoloration (neck and abdomen).

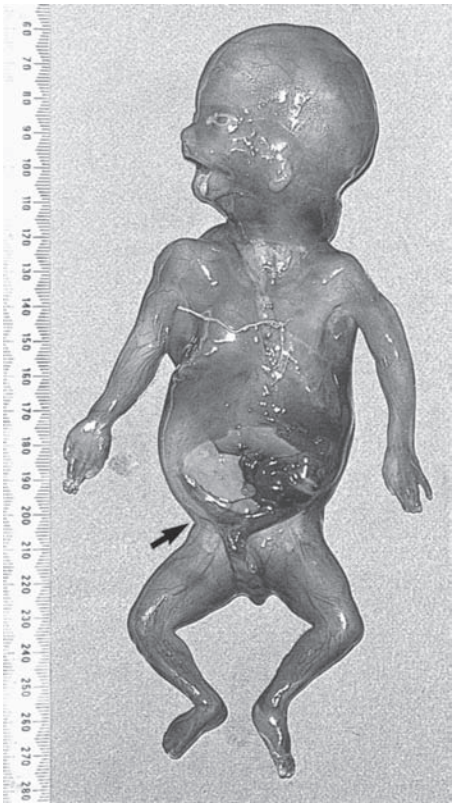


Figure 10.3. Macerated and autolyzed fetus with inguinal masses (arrows) of autolyzed cerebral tissue; the cranial vault is collapsed.

autolyzed brain tissue into the spinal cord and along the spinal nerve roots into the retropleural and retroperitoneal areas may produce tumorous masses of neural tissue, which should not be mistaken for a neurogenic neoplasm (Kalousek et al. 1988) (Fig. 10.3).

When the fetus is retained in utero for 7 to 10 days, further changes in color occur, from purple to brown discoloration. During retention of the fetus for several weeks, a fading of color to yellow–gray will occur. Progressive loss of fluid from the fetus eventually results in the formation of "fetus papyraceous." Dehydration results in shrinkage and compaction of tissues and organs. Very occasionally an extrauterine pregnancy may be retained for years forming so-called lithopedion (Greek *lithos,* stone, and *paidion,* small child), a calcified fetus retained in the abdominal cavity (Elechi and Elechi 1995).

Limitations of Examination

Even in the presence of advanced maceration, as full and detailed a necropsy as possible should be performed. Some measurements and weights may be altered by the process of maceration; nevertheless, when used together, they may help in evaluation of fetal growth.

Body weight and external measurements should be recorded irrespective of the degree of maceration. Connective tissue autolysis may allow stretching and elevate both the crown–rump and crown–heel lengths. In this situation, the foot length still remains the least altered parameter and supplies a reliable marker for gestational assessment.

Overlapping of the cranial vault bones may invalidate the head circumference measurement, but if taken with care and used in conjunction with foot length, it may add extra information to the assessment of fetal growth.

In a long-standing IUD, body and organ weights will not be accurate. Organ weight ratios rather than absolute values should be used.

Softening of tissues may produce difficulties in the dissection of the internal organs, particularly when a congenital malformation is suspected as in, for example, congenital heart defect. Brief formalin fixation (for 24 to 48 hours) will produce

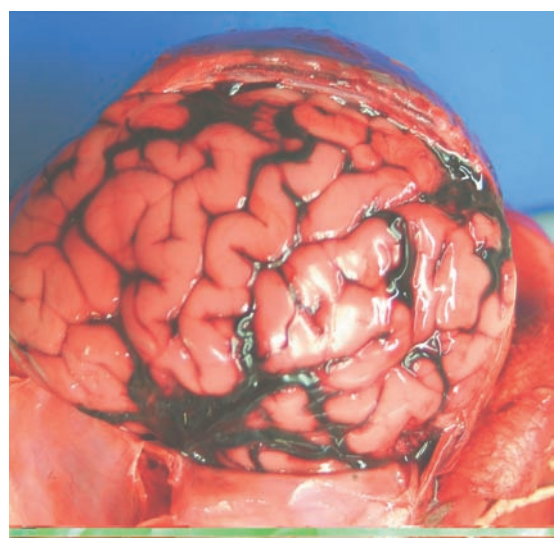


Figure 10.4. Macerated stillbirth: the skull has been opened to expose the surface of the cerebral hemispheres. The complexity of the gyral pattern can aid assessment of gestational age (consistent with 40 weeks' gestation).

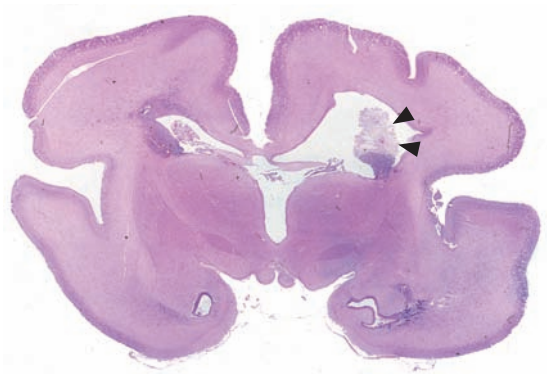


Figure 10.5. Macerated stillbirth at 27 weeks' gestation. The brain was suspended and fixed in formalin for 4 weeks prior to dissection (with permission for retention). This coronal section at the level of the foramen of Monro reveals old intraventricular hemorrhage (arrowheads).

some hardening of the tissue and permit adequate dissection and demonstration of structural abnormality.

Organs most severely affected by autolysis in macerated stillbirths are usually those from the abdominal cavity (pancreas, liver, spleen, adrenal glands) and brain. The presence of proteolytic enzymes, abundant in liver tissue, accounts for their rapid and extensive autolysis. Fetal brain with its high water content is very soft and friable, even in the fresh state. In the presence of maceration and advanced autolysis, removal of the brain may be very difficult. Despite rapid softening of tissues, the gyral pattern of the cerebral hemispheres is retained, and careful inspection of the gyri after opening of the skull and dura may help in assessment of gestational age, particularly important when there is intrauterine growth restriction (Fig. 10.4).

Removal of the brain into a formalin-filled container has been advocated to minimize trauma associated with handling. A soft cloth can also be used to transport and suspend the brain in a suitable container for fixation with good structural preservation (Fig. 10.5).

Histological Examination

Even in cases of severe maceration, histological sampling of all internal organs should be undertaken. Histological examination can help in the assessment of gestational age at the time of death, the mode of death (sudden or associated with prolonged stress), and the presence of viral infection or maternal diabetes.

Routine hematoxylin and eosin stain may not be helpful as, with progressive autolysis, there is increased loss of nuclear detail. However, connective tissue stains (trichrome or reticulin stain) may outline the architectural detail and may enable assessment of organ development and identification of pathological abnormalities. In the abdominal cavity the histological detail of the liver and spleen is lost quickly, but kidneys and adrenal glands retain their details for longer. A frozen section of the adrenal gland facilitates the assessment of the distribution of the intracellular lipid and supplies information about the mode of death (Becker and Becker 1976). In the investigation of nonimmune hydrops immunocytochemistry can usefully be performed, even on autolyzed tissue aiding the identification of viral inclusions (Wright et al. 1996).

Additional Investigations

Microbiological Studies

There is conflicting evidence about the significance and frequency of infection in cases of IUD. Infection is less frequent in macerated stillbirths compared to fresh stillbirths and neonatal deaths (Keeling 1987).

Amniotic fluid infection is listed as one of the common causes of fetal death in the U.S. Collaborative Perinatal Project (Naeye 1977). Lessing et al. (1989) described a variety of positive bacterial cultures in 28 out of 57 fetuses delivered after IUD. The most commonly isolated organisms were *Escherichia coli* and *Proteus mirabilis*. These findings were not confirmed by histological examination of the membranes and fetal organs and are of questionable significance. Moyo et al. (1996) documented the presence of acute chorioamnionitis in 79% of stillbirths of African mothers, with *E. coli* and group B streptococcus being the most frequent pathogens. In 9% of such cases an inflammatory reaction was present in the chorionic plate vessels, indicating antemortem infection.

Genital mycoplasmas are present in 8.3% of stillbirths and neonatal deaths, significantly more often associated with acute chorioamnionitis and

funisitis (Madan et al. 1988). However, isolation of genital mycoplasmas from fetal tissues is not associated with increased incidence of IUDs.

In macerated stillbirths routine bacteriological and viral cultures are not justified in every case but should be performed in cases where clinical information and pathological findings are suggestive of infection. These would include not only cases with obvious signs of infection (opacity and necrosis of the placental membranes) but also cases with severe intrauterine growth restriction and nonimmune hydrops. In the last two conditions intrauterine viral infection should be considered. B18 parvovirus infection is not usually associated with stillbirth and has been documented in only 0.6% of deaths occurring before 20 weeks (Enders at al. 2004). Congenital echovirus infection (echovirus type 33 and 27) might be suspected in the fetus with marked hemorrhagic necrosis, particularly in the peripheral muscles, and viral cultures should be undertaken in such cases (Garcia et al. 1990).

Chromosome Analysis

Chromosome examination in macerated stillbirths is fraught with difficulty but should be attempted in cases with congenital defects, severe intrauterine growth restriction, or nonimmune hydrops (Pitkin 1987).

Although 30% to 35% of stillbirths may have notable congenital defects (Mueller et al. 1983), only 5% to 11% of macerated stillbirths will have chromosomal abnormalities (Carey 1987; Magani et al. 1990). Chromosomal abnormalities found in macerated stillbirths were of the same type as those present in neonatal deaths but were twice as common (11.5% compared to 5.6%) (Alberman and Creasy 1977).

In slightly macerated stillbirths lung and diaphragm cultures should be attempted, but very often the only viable tissue left is that of the placenta. A small sample of amnion (1 to 2 cm^2) should be removed with a sterile forceps, placed in a sterile container with or without the culture medium, and transported immediately at room temperature. Successful culture from such material varies considerably among laboratories, and published data on abnormal karyotypes should be interpreted with caution. Sutherland and Carter (1983) obtained successful chromosomal analysis from 54% of macerated stillbirths and detected abnormalities in 19% of them. In cases with strong suspicion of chromosomal abnormality when cultures fail, use of in-situ hybridization may detect major chromosomal anomalies (Drut et al. 1992).

Radiographic Examination

Radiographic appearances of the fetus are not affected by maceration. In fact, the laxity of joints enables easy alignment of the upper and lower limbs. Radiography may contribute to assessment of gestational age and provides an accurate record of any suspected skeletal abnormality.

The usefulness of radiological investigation is well documented. Out of 400 fetuses examined in the study by Kalifa et al. (1989), additional information was obtained from postmortem radiographic examination in 34.5% of cases; Pauli et al. (1994) showed that 22% of radiographs of stillborn fetuses yielded diagnostically important abnormalities.

Ultrasound

In cases where parents are unwilling to consent to a full necropsy, permission may be obtained for ultrasound scanning examination, or ultrasound-guided needle biopsy in cases of suspected metabolic disorders (Furness et al. 1989).

Examination of the Placenta

Placental examination should be considered an integral part of the autopsy on a macerated stillborn infant. After death in utero the placenta remains viable and undergoes progressive morphological changes that commence 24 hours after fetal death and are fully established 5 or 6 days later (Fox 1978; Genest 1992).

Macroscopically recognizable changes associated with IUD include maternal flow infarction (Benirschke and Driscoll 1967). In cases of IUD of a few weeks' duration the placental tissue becomes firm and whitish in color with progressive reduction of the placental volume. Extensive subchorial thrombus formation, also called Breus's mole, may be apparent following IUD, possibly related

to sudden slowing of the blood flow through the intervillous space.

Following the cessation of fetal circulation, several characteristic postmortem changes are observed on histological examination of the placental villi. The villi show an increased number of syncytial knots. The number of cytotrophoblastic cells is increased and thickening of the basement membrane is observed. The basement membrane of the trophoblast may become mineralized in the first 5 days after death in utero (Wigglesworth 1984). Many of the villi show prominent stromal fibrosis, capillary collapse, and fibromuscular sclerosis of the fetal stem arteries with narrowing and full obliteration of their lumen.

Occasionally, villous edema may be seen, and aggregates of placental dystrophic calcification observed. Calcification is as common in fresh stillbirths as in liveborn infants, but in placentas from IUDs with a longer death to delivery interval there is a progressive loss of calcium rather than excessive accumulation (Fox 1978).

Several postmortem changes are also observed in the umbilical cord. Edema and dull red discoloration, particularly of the fetal end of the cord, are frequently seen in macerated stillbirths. Excessive twisting of the cord has been described in relation to increased fetal flotational movements after IUD (Edmonds 1954) (Fig. 10.6). Usually, it is more prominent at the fetal end of the cord and it should be distinguished from antemortem twisting, which remains permanent after the separation of the fetus and placenta (Browne 1925). Histological examination shows congestion, thrombosis, and edema at the site of antemortem coiling, and these features are absent in cases of postmortem twisting. Edmonds (1954) suggested that cord stricture is another postmortem artifact due to maceration of the cord close to the fetal end. The majority of the strictures have been described in stillborn infants and probably do not antedate fetal death in utero (Weber 1963; Gilbert and Zugibe 1974).

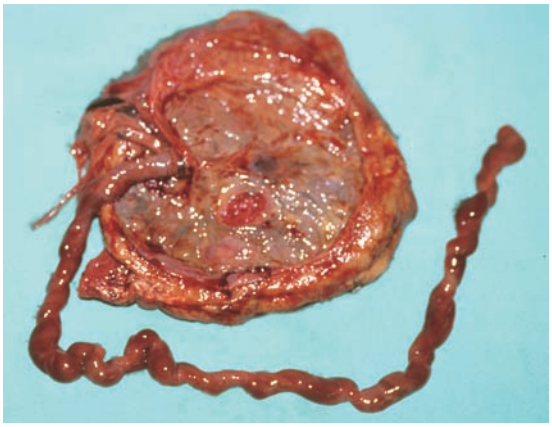


Figure 10.6. Dark red discoloration and excessive twisting of the cord in a macerated stillbirth.

The presence of true tight knots should be clearly distinguished from "false knots" represented by local dilatation of umbilical vessels or focal thickening of Wharton's jelly. A previously loose knot might become tightened during delivery. Any cord with a tight knot should be carefully examined for the presence of marked and permanent grooving on the surface of the cord at the site of the knot, loss of Wharton's jelly, and constriction of the vessels. The presence of thrombus in the lumen of the vessels and iron-laden macrophages in the Wharton's jelly would support the observation of antemortem formation of the knot. Very rarely a tight cord knot may contribute to intrauterine demise, particularly in association with excessive length of the cord (greater than 80 cm; see Chapter 2). The significance of knots in the cord was disputed by Browne (1925), although Sornes (2000) found a 10-fold increase in IUD in the presence of a cord knot. Machin at al. (2000) suggested that abnormal coiling, either over- or undercoling, contributed to 37% or 29%, respectively, of stillbirths.

Cord and placental complications have been suggested as chief contributory factors in 10% to 20% of IUDs (Pitkin 1987) and placental abnormalities as major contributing factor in 58% of stillbirths (Magani et al. 1990).

Significant pathological abnormalities identified on macroscopic examination of the placenta include retroplacental hemorrhage, placental infarction, and opacity of the placental membranes indicating infection. However, retroplacental hemorrhage and infection are not as frequent in macerated stillbirths as they are in the placentas from fresh stillborn infants. The margination of maternal neutrophils in the subchorionic tissue of the placental disk in response to a dead fetus should be distinguished from chorioamnionitis due to ascending infec-

Figure 10.7. Slices of the placenta from a stillbirth showing numerous areas of infarction of different duration.

tion. Infarcts occupying more than 10% of the placental cross-sectional area should be considered as an indication of compromised uteroplacental blood flow (Fox 1978) (Fig. 10.7). Massive areas of infarction may be observed in placentas from mothers with lupus anticoagulant and anticardiolipid antibodies.

Placentas from hydropic stillborn infants are usually heavy, pale, and edematous. Microscopically, numerous nucleated red cells may be detected in the fetal blood vessels, indicating severe anemia.

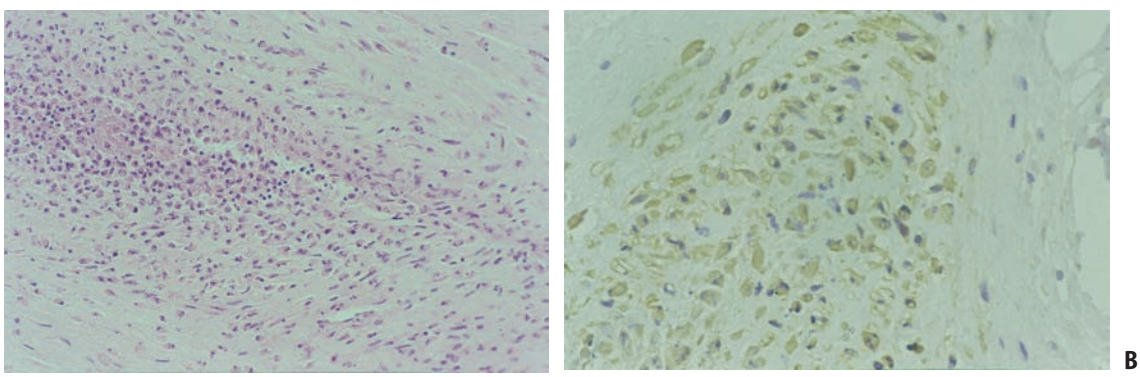
Hemorrhagic endovasculitis with thrombosis of the chorionic vessels, extravasation, and fragmentation of red blood cells was found in 17% of the placentas from stillborn fetuses (Sander and Stevens 1984). The significance of this lesion is uncertain. Another recently described artifact related to fetal retention is umbilical cord pseudovasculitis, in which degenerating smooth muscle cells closely resemble neutrophils, which may lead to erroneous diagnosis of infection (Genest et al. 1997) (Fig. 10.8).

Pathological Findings in Macerated Stillbirths

Incidence

Approximately 1% of pregnancies are complicated by death of a fetus after 20 weeks (Pitkin 1987). The incidence of pathological abnormalities in macerated stillbirths is difficult to ascertain, as frequently only broad categories are described and only selected findings are quoted. The search for etiological factors may overshadow other pathological findings, which may be considered of no particular significance.

A direct cause of death is found in only 50% of stillbirths. Slightly higher figures were quoted by Brandt and Holmskov (1990), in whose study the diagnosis was established in 78% of prenatal deaths. Studies by Hovatta et al. (1983) and Magani et al. (1990) quoted anoxic changes as the most



A B

Figure 10.8. (A) Autolyzed umbilical cord with degenerating cells resembling neutrophils. (B) Immunohistochemistry for smooth muscle actin shows positive staining in the cytoplasm of degenerate cells, consistent with a smooth muscle origin (staining for macrophage and common leucocyte antigen were negative).

common finding in stillbirths but did not distinguish between antepartum and intrapartum deaths. Histological examination was not performed in all of their cases. Pauli and Reiser (1994) demonstrated that in 40% of stillbirths a specific cause of death was identified and in nearly a quarter of these an identifiable maldevelopmental disease process was found. Gardosi et al (2005) claimed that an application of the classification system identifying all relevant clinical conditions at the time of death may provide a better explanation of stillbirths. Intrauterine growth restriction was present in 43% of cases reviewed by these authors.

Mode of Death

Acute and chronic modes of death can be established in stillbirths by careful evaluation of external appearances of the organs, their weights, and the weight ratios, and from the histological examination.

In an acute mode of death (death occurring within 24 hours of the initial incident), asphyxial petechial hemorrhages are detected on the surface of the thoracic organs (Fig. 10.9). Abdominal organs are very congested but with normal weight ratios. Examination of the adrenal gland plays a crucial role in the assessment of mode of death (Becker and Becker 1976). Frozen sections stained with oil red O or Sudan black demonstrate the pattern of lipid distribution in this organ. In the acute mode of death of a previously uncompromised fetus, there is absence of lipid in the fetal cortex with preservation of lipid in the definitive cortex. A similar distribution of fat is seen in the normal adrenal gland. In chronic IUD, with the disease process lasting for at least 3 weeks, the fetal zone of the adrenal gland demonstrates accumulation of lipid, and in such cases placental pathology frequently suggests chronic fetal hypoxia.

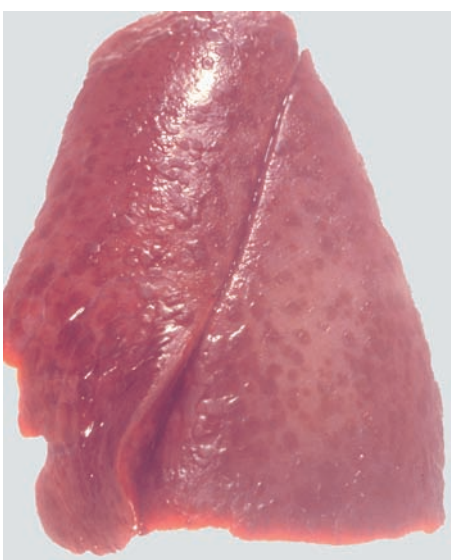


Figure 10.9. Lungs from a stillbirth: many petechial hemorrhages are present on the visceral pleura. This was accompanied by marked congestion of the abdominal organs.

Delprado and Baird (1984) reported a very similar pattern of lipid deposition in the adrenal gland and suggested that any lipid deposition in the fetal cortex was the marker of chronic fetal hypoxia. Similar changes were observed by Claireaux (1950) in the adrenal glands of hydropic infants from rhesus-incompatible pregnancies.

Asphyxia

In a study from Scotland, half of fetal deaths were attributed to asphyxia (Whitfield et al. 1986). Pathological signs of asphyxia include thoracic visceral and parietal serosal petechial hemorrhages; squamous debris aspirated into the distal airways; splanchnic hemorrhagic necroses in liver, spleen, kidneys, and adrenals; and cerebral subependymal/intraventricular hemorrhages (Figs. 10.10 and 10.11). In a fetus retained after death in utero for longer than 3 days, petechial hemorrhages gradually fade away.

Abruptio placentae is one of the most commonest causes of asphyxia and contributes to up to one third of fetal deaths (Kochenour 1987). Polyhydramnios, trauma, and short umbilical cord are traditionally thought to be associated with abruptio placentae. This event occurs more commonly in hypertensive mothers (Abdella et al. 1984).

Antepartum hemorrhage due to placenta previa is not a common finding in macerated stillbirths. Naeye (1978) reported its presence in 11 stillbirths out of 53,518 pregnancies.

Cord accidents (true knots, cord prolapse, and cord around the neck) accounted for 48% of fetal asphyxia in Morrison and Olsen's (1985) study,

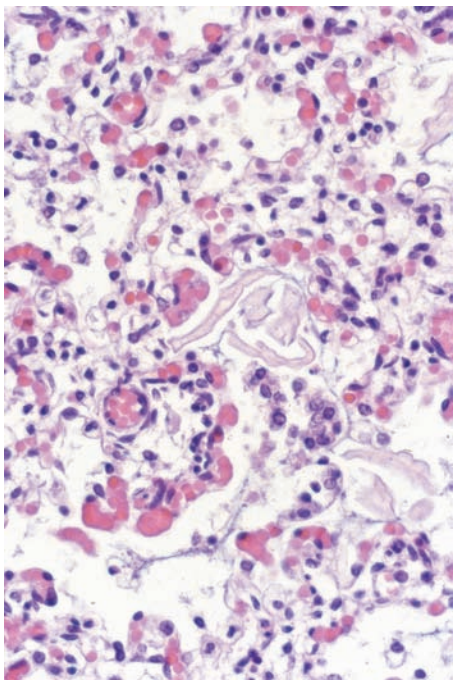


Figure 10.10. Section of the lung revealing squamous and granular debris filling the terminal airways, consistent with meconium aspiration in an asphyxial episode in utero.

while an account by Magani et al. (1990) supports the view that mechanical factors, including cord accidents, are not very common in stillbirths (12% overall incidence). Thrombosis of umbilical cord vessels and cord hematomas, although rare, are recognized causes of death in utero (Ibbetson et al. 1994). Umbilical cord stricture has been suggested by Sun et al. (1995) as an underrecognized cause of midtrimester fetal deaths.

Cerebral Pathology

Ischemic lesions in the brain, including intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL), have been documented in fetuses dying in utero before the onset of labor (Bose 1978; Taylor and Roessmann 1984). Approximately 6% of stillbirths are noted to have IVH at autopsy (Harcke 1972). Detailed histological examination of brains from stillbirths shows features of ischemia and prenatal damage much more often than initially anticipated. Sims et al. (1985) demonstrated IVH and gliosis in 25 of 433 consecutively examined stillbirths; 36% of cases with cerebral pathology were associated with the presence of chorioamnionitis.

Squier and Keeling (1991) described the presence of ischemic and hemorrhagic brain damage in 40% of stillborn infants. The most frequent lesion found in this study was diffuse white matter ischemia, seen in 26% of cases. This abnormality was only detected in brains of fetuses of more than 27 weeks' gestation. Pontosubicular neuronal necrosis, comprising neuronal necrosis with karyorrhexis in pontine nuclei and in the subicular segment of Ammon's horn, has been observed in 40% of brains from stillbirths by Skullerud and Skjaeraasen (1988). This lesion was associated with maternal diabetes and intrauterine growth restriction. More recently, Becher et al. (2006) described both recent and established hypoxic brain damage in antepartum stillbirths. Brain damage was associated with pregnancy-induced hypertension, prematurity, and low placenta weight. Hypoxic damage appeared to be an ongoing process in many babies with a high correlation between recent and established injury.

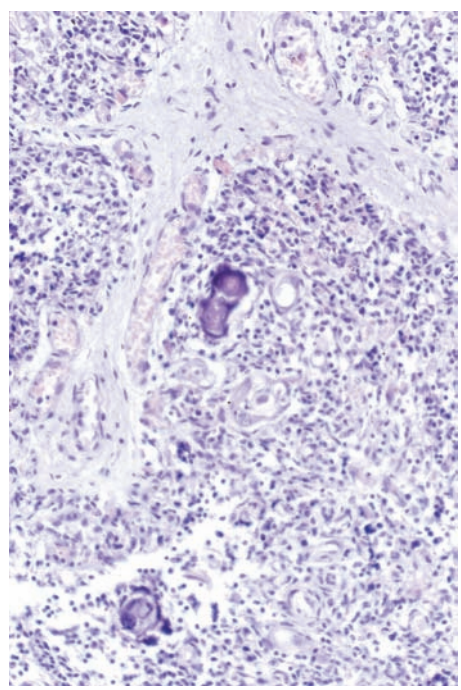


Figure 10.11. Thymus displaying loss of cortical lymphocytes with exaggerated separation of thymic lobules indicating exposure to prolonged intrauterine stress.

Malformations

Major congenital defects are not seen as frequently in stillbirths as they are in neonatal deaths. Machin (1975) described lethal malformations in 11% of 185 antepartum deaths but were found in 36% of antepartum stillbirths by Meagher and Boyd (1993). In a study of 253 macerated stillbirths examined in Oxford, malformations were found in 13% (Table 10.1). Many of these cases had malformations in several systems and, in many, prolonged uterine existence would not have been possible without surgical intervention. The most common major congenital anomalies include central nervous system (CNS) anomalies, congenital heart defects, and major renal anomalies.

The recognition of a combination of defects commonly associated with fetal mortality may provide an accurate cause of death, for example, holoprosencephaly, congenital heart defect, and facial anomalies in trisomy 13, or dysmorphism and growth restriction in triploidy (Fig. 10.12). However, the presence of minor anomalies with their overall frequency of 1% to 3% may not by itself indicate a malformation syndrome or provide a cause of death, but may lead to a diagnosis of a common disorder such as trisomy 21. The rate of minor abnormalities is increased in stillbirths compared with the general population. Single umbilical artery has been documented in 3.1% of stillbirths while in the general population it is of the order of 0.5% (Heifetz 1983).

The association of malformations and intrauterine growth restriction is well known. They are seen in 5% to 15% of growth-restricted infants (Chiswick 1985). The Oxford study documented an increased number of malformations in infants

Table 10.1. Malformations among 253 macerated stillbirths (Keeling 1987)

System affected	Details of malformation	Number of cases
CNS	Hydrocephalus, exomphalos, tracheoesophageal fistula, esophageal atresia	
	Hydrocephalus, meningomyelocele, VSD	
	Hydrocephalus, periventricular calcification	
	Hydrocephalus, exomphalos, renal agenesis, sirenomyelia	8
	Hydrocephalus, exomphalos	
	Hydrocephalus, ambiguous genitalia	
	Hydrocephalus	
	Anencephalus	
Cardiac	Cardiac rhabdomyomas, hydrops fetalis	
	Cardiac rhabdomyomas	
	Atrioventricular canal defect, asplenia syndrome, hydrops fetalis	
	Atrioventricular canal defect	
	Hypoplastic left heart, gross ascites	
	Interrupted aortic arch, aortopulmonary window, Klippel-Feil anomaly	14
	TGA, VSD, exomphalos, cleft palate	
	TGA, VSD, cleft lip and palate, minor anomalies	
	TGA, polydactyly, flexion deformities	
	Cardiac conduction system anomaly, hydrops fetalis	
	VSD (3)	
	Sinus venosus atrial septal defect	
Renal	Renal hypoplasia, hydrops fetalis	5
	Renal agenesis (3)	
	Bilateral polycystic kidneys	
Gastrointestinal	Exomphalos, volvulus, intestinal infarction	3
	Esophageal atresia, tracheoesophageal fistula	
	L. diaphragmatic hernia, hydrops fetalis	
Skeletal	Thanatophoric dysplasia	2
	Absent radii and thumbs, flexion deformities	
Respiratory	Adenomatoid malformation lung, hydrops fetalis	1
Total		33

TGA, transposition of great arteries; VSD, ventricular septal defect.

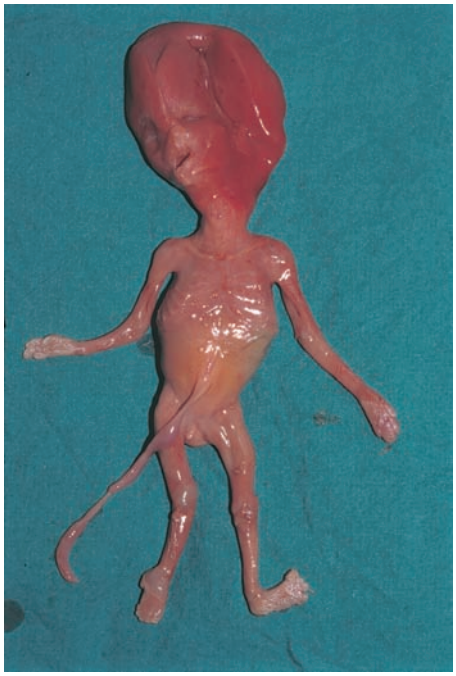


Figure 10.12. Macerated stillbirth with disproportion of head and trunk, talipes, and bilateral syndactyly 3/4. Culture of placental amnion revealed triploid karyotype.

of low birth weight among 253 macerated stillbirths (Table 10.2).

Stillbirths with congenital malformations have a higher frequency of chromosomal abnormalities (5% to 10%). The most common of these include trisomies 21, 18, and 13 and monosomy X. Stillbirths with these chromosomal disorders may show hydrops fetalis; particularly common are monosomy X and trisomy 21. Uniparental disomy, in which both homologous chromosomes are inherited from one parent, is suspected to contribute to some of the stillbirths (Gilbert-Barness 1997). This may require DNA studies of both parents and their offspring.

Placental Abnormalities

Infarction is one of the commoner placental abnormalities detected on macroscopic examination. It was shown in 15% of placentas from stillbirths (Hovatta et al. 1983). These are usually multiple small areas of infarction, sometimes mixed with larger and recently acquired infarcts.

Table 10.3 shows the pathological abnormalities identified in fetus and placenta in 220 normally formed macerated stillbirths (Keeling 1987). Placental abnormalities, including retroplacental hemorrhage, infarction, and tight umbilical cord knots, were twice as common in stillbirths with signs of asphyxia as in those without such signs. Although not all the placental abnormalities present in Table 10.3 could be causally related to IUD, retroplacental hemorrhages, infarction, and tight knots in the cord were considered as likely causes of death.

Antepartum hemorrhage with or without placental abruption is recognized as a single largest known cause of stillbirth, contributing to up to 17% of deaths (Gilbert-Barness 1997).

Maternal Disorders Associated with Stillbirths

One of the major factors influencing the increased risk of stillbirth is a history of chronic maternal disease such as hypertension or diabetes. Nevertheless, even increased maternal age (>30 years) or a very young maternal age (<20 years) are associated with higher risk of stillbirths (Naeye 1983; Brans et al. 1984).

Maternal hypertension is a major cause of perinatal mortality (Lin et al. 1982) with a higher risk of fetal death when eclampsia is superimposed (Sibai et al. 1984). Abruptio placentae is often seen in pregnancy-induced hypertension with a three

Table 10.2. The 253 macerated stillbirths of Table 10.1 grouped by necropsy weight and form (Keeling 1987)

	1000 g	1500 g	2000 g	2500 g	3000 g	3000 g	Total
Malformed	11	11	3	6	0	2	33
Normally formed	38	37	48	32	31	34	220
Signs of asphyxia	13	14	20	18	19	24	108
No asphyxia	25	23	28	14	12	10	112
Total	49	48	51	38	31	36	253

Table 10.3. Pathological abnormalities in 220 normally formed macerated stillbirths (Keeling 1987)

	Signs of asphyxia $n = 108$	No asphyxia $n = 112$	Total
Fetus			
Intrauterine growth retardation	26	7	33
Hydrops with ascites	4	6	10
Renal vein thrombosis	5	1	6
Renal artery thrombosis	1	—	1
Renal cortical necrosis	1	—	1
Inflammatory infiltration of myocardium and calcification	2	2	4
Calcification of myocardium	1	—	1
Inflammation in several organs	—	1	1
Hepatosplenomegaly	—	1	1
Thyroid enlargement	—	1	1
Cardiac teratoma	—	1	1
Neuroblastoma in situ	1	—	1
Flexion deformities	2	1	3
Mild hydronephrosis and hydroureter	—	1	1
Uterus unicornis	1	—	1
Dysmorphic features	2	—	2
Single umbilical artery	—	1	1
Total	46 (43%)	23 (21%)	69 (32%)
Placenta and cord			
Retroplacental hemorrhage	20	11	31
Hemorrhage and infarction	33	20	53
Hydrops	2	4	6
Chorioamnionitis	6	2	8
Funisitis	2	—	2
Circummarginate placenta	2	—	2
Tight cord knots	3	1	4
Cord enlargement	6	1	7
Placental artery aneurysm	1	—	1
Iatrogenic vasculitis and thrombosis	—	1	1
Total	75 (68%)	39 (37%)	114 (52%)

to four times increased risk of intrauterine death (Abdella et al. 1984).

Maternal diabetes is another important cause of perinatal mortality, also contributing to increased incidence of congenital malformations (four times higher than in the background population) (Hawthorne et al. 1997). It has been shown that the placental blood flow in pregnancy complicated by diabetes is decreased by 35% to 45% (Nylund et al. 1982). Mimouni et al. (1988) suggested that the increased risk of perinatal hypoxia in insulin-dependent diabetic mothers might reflect a left shift of the blood-oxygen dissociation curve caused by increased concentrations of glycosylated hemoglobin, in effect producing a decreased availability of the oxygen to the fetus. More than 18% of women with unexplained stillbirth had an abnormal glucose tolerance test result but did not meet the criteria for gestational diabetes in the study by Lau and Li (1994). Distal villous immaturity may be seen in association with gestational diabetes, attributed to hyperinsulinemia with an increase in insulin-like growth factor leading to accelerated growth of connective tissue and capillaries in the terminal villi (Kraus et al. 2004). Similar changes may occasionally be seen without the underlying maternal diabetes, and this defective placental maturation could be responsible for 2.3% of fetal deaths after 35 weeks of gestation (Stallmach et al. 2001).

Connective tissue disorders in the mother are associated with a high risk of fetal death and recurrent spontaneous abortion in the first and second trimester. Nilsson et al. (1975) documented a very high incidence of pregnancy loss in women with lupus anticoagulant. Lupus anticoagulant is an antiphospholipid antibody interfering with coagulation pathways and leading to thrombotic events and vasculopathy involving the placental decidual vessels and resulting in infarction. It has been suggested that lupus anticoagulant and raised levels of anticardiolipin antibodies are each independently associated with fetal loss, and they should be sought even in apparently healthy mothers following fetal loss (Creagh et al. 1991).

In fetal deaths associated with antiphospholipid antibodies, demise appears to be a consequence of the uteroplacental vascular damage (Sebire et al. 2003). Another mechanism that contributes to a small proportion of fetal deaths to mothers with connective tissue disorders is development of complete heart block in the fetus. There is a strong association between a complete congenital heart block and maternal connective tissue disease that is present or subsequently develops in 30% of mothers of affected infants. Extensive endocardial damage with fibrosis in the region of the atrioventricular node has been shown in such cases (Steier et al. 1987).

Massive fetomaternal hemorrhage is rare, demonstrated in 0.04% of all births (Laube and Schauberger 1982) but it is probably an underestimated cause of fetal death, particularly among

macerated stillbirths. Small fetomaternal hemorrhages occur in up to 39% of otherwise normal pregnancies, but these hemorrhages are of no significance. Clinically significant hemorrhage occurs when 20% of fetal blood volume is lost (Owen et al. 1989). Even with significant fetomaternal hemorrhage, much in excess of the calculated fetal blood volume, postmortem findings may not be very striking, with occasional cases showing pallor of skin and mucous membranes (Fay 1983).

Certain maternal occupations carry a higher risk of stillbirth. Maternal work in the rubber, plastics, and textile industries, as well as lead exposure, increase the risk of stillbirth, preterm delivery, and small-for-gestational-age infants (Savitz et al. 1989).

Maternal smoking is a well-recognized risk factor for fetal death. High cigarette consumption was identified as the strongest risk factor for intrauterine death in growth-restricted stillbirths (Frøen et al. 2002). It is estimated that if all pregnant women stopped smoking, the number of fetal and infant deaths would be reduced by 10% (Kleinman et al. 1988). Naeye (1980) described an increased incidence of abruptio placentae and placenta previa in smoking mothers. Maternal drug abuse, particularly cocaine, has been associated with placental abruption due to vasoconstrictive effect (Chasnoff et al. 1985).

Stillbirth and Multiple Gestation

The decreasing incidence of multiple births in England and Wales was reversed in the 1980s, when introduction of fertility drugs that induce ovulation and, more recently, in vitro fertilization, have contributed to increased multiple births. Mortality in multiple births is about five times higher than for singletons (Pauli and Reiser 1994). Twins were three times as common among macerated stillbirths (18 of 253) examined in Oxford (Keeling 1987). Intrauterine death of one of twins may not be recognized for some time, although this is less likely with ready access to ultrasonography. Cerebral and renal cortical necroses have been described in a surviving twin after death of the co-twin (Moore et al. 1969). The most common cerebral lesion found in the longer surviving twin is widespread periventricular leukomalacia. The hazards of multiple pregnancy are discussed further in Chapter 12.

Conclusion

Necropsy on a macerated stillbirth is no longer recognized as an unnecessary procedure, but unfortunately, despite clear benefits, there is a decline in autopsy rates in many countries (Kock et al. 2003). The Danish study of Kock et al. (2003) showed that in 9% of stillbirths autopsy results changed the final diagnosis and in 22% additional important information was obtained. A previous British study has shown that clinically important differences between clinical and pathological diagnoses were present in 36% of stillbirths (54 of 150 cases) (Porter and Keeling 1987).

The necropsy on a macerated stillbirth may be aesthetically unpleasant but this should not deter the pathologist from as full and as diligent an examination as possible. This should include histological examination, radiology and cytogenetic studies, and microbiology in some cases; clinical diagnosis was reached after histological examination of the tissues in 20% of stillbirths (Porter and Keeling 1987).

During postmortem examination of macerated stillbirths, abnormalities that are not causally related to IUD will be detected; this may have clinical importance. Even negative findings after carefully performed necropsy are useful in future parental counseling.

Pathological investigation of stillbirths plays an important role as improvement in the quality of information obtained from necropsies is an essential component of any strategies aiming at reduction of perinatal mortality such as confidential inquiries into maternal and child health.

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11
Prematurity

Andrew J. Lyon

In industrialized countries, preterm birth is responsible for 70% of neonatal mortality and 75% of neonatal morbidity, and contributes to long-term neurodevelopmental problems, pulmonary dysfunction, and visual impairment. In the United Kingdom, the perinatal mortality rate (stillbirths from 24 weeks' gestation and first week neonatal deaths) is now less than 10 per 1000 total deliveries. In Scotland there has been a fall in the total number of live births from 60,051 in 1995 to 52,432 in 2003. In the same time period, the perinatal mortality rate has fallen from 9.6 to 8.0/1000 total births, but there has been no real change in the causes of perinatal death as shown in Tables 9.1 and 9.2 in Chapter 9. In the "unexplained <2500 g" group in Table 11.1, premature labor or preterm rupture of membranes is present in one third of the cases. In Table 11.2, problems of prematurity are second only to congenital abnormalities as the cause of neonatal death. The latter may be influenced by selective termination, but any further decrease in perinatal mortality will rely on our ability to reduce prematurity and its problems. However, the proportion of babies delivering preterm (before 37 completed weeks' gestation) remains constant at around 6% of all deliveries. Preterm babies contribute significantly to the workload of a neonatal unit. In Edinburgh, babies born alive before 32 weeks' gestation are 3% of the total live deliveries. They make up only 19% of the admissions to the neonatal unit but contribute 85% of the intensive care days and 52% of the total treatment days. The problems of the preterm infant are directly related to gestational age, as this determines the functional immaturity of the systems of the body. Birth weight is related to outcome because of its close relationship to gestational age but also, at any gestation, survival is better among heavier infants (Piper et al. 1996). The poorest outcome is seen in infants with birth weight more than two standard deviations from the mean for that gestation, that is, those with marked intrauterine growth restriction.

Causes of Prematurity

The inability to reduce the proportion of premature deliveries is at least in part due to a failure to determine the cause(s) of preterm labor.

Epidemiology

The epidemiological associations of prematurity are those of poverty and social disadvantage, the risks almost doubling when "unskilled" are compared with "professional" classes (Macfarlane et al. 1988). Preterm delivery is more common among single unsupported women, but this effect may just be an indicator of some more complex factor, such as social support (Lumley 1993). There is an association with late attendance for antenatal care or not receiving antenatal care at all, but those delivering early may not have had as much opportunity to attend (Tyson et al. 1990). Women under 20 years of age have an increased rate of preterm delivery with increasing parity, while in all other age groups primiparity is associated with a higher rate of preterm birth compared with subsequent pregnancies.

Table 11.1. Stillbirth and neonatal deaths by obstetric classification (Cole et al. 1986)

	1995	2003
Congenital anomalies	16%	16%
Pregnancy-induced hypertension	8%	4%
Antepartum hemorrhage	16%	13%
Trauma	3%	3%
Maternal disease	10%	6%
Miscellaneous	3%	1%
Unexplained <2500 g	28%	37%
Unexplained ≥2500 g	16%	19%

Source: Scottish Perinatal and Infant Mortality and Morbidity Report (SPMMR), 1995 and 2003, ISD Scotland.

Smoking in pregnancy is associated with an excess of preterm deliveries, particularly in those who smoke more than 20 cigarettes a day (Meyer and Tonascia 1977). There is an increase in those without an identifiable cause ("idiopathic preterm labor") and also a dose-dependent increase in risks of preterm birth due to preterm premature rupture of membranes and late pregnancy bleeding (Kyrklund-Blomberg et al. 2005). Cigarette consumption is increased among the socially deprived and the unemployed, and it is possible that many of the effects of stressful life events or poor psychosocial circumstances on pregnancy outcome may be mediated through smoking (Cliver et al. 1992). Intensive efforts during pregnancy, using good-quality motivational interviewing, have unfortunately not been shown to reduce maternal smoking (Tappin et al. 2005).

The proportion of births before 37 weeks has changed little despite socioeconomic improvements and better obstetric care. The effect of the classic epidemiological associations of preterm birth is small compared with factors in the prior reproductive history and with medical and obstetric complications of the current pregnancy.

Table 11.2. Neonatal death by pediatric classification (Hey et al., 1986)

	1995	2003
Congenital anomalies	38%	35%
Anoxia/birth trauma	10%	13%
Lung immaturity	14%	17%
Respiratory distress syndrome	11%	10%
Intracranial hemorrhage	1%	1%
Other hemorrhage	1%	0%
Infection	13%	13%
Other pediatric factors	10%	7%
Unexplained	2%	4%

Source: Scottish Perinatal and Infant Mortality and Morbidity Report (SPMMR), 1995 and 2003, ISD Scotland.

Prior Reproductive History

There is a relationship between previous abortions and preterm delivery that cannot be explained by confounding sociodemographic factors (Lumley 1993). There is also a dose–response relationship between the number of unsuccessful pregnancies and the prevalence of preterm birth, which suggests some common factor, possibly genetic or relating to implantation, causing both outcomes.

Preterm delivery rates are increased where a woman has already had a premature baby and the risk increases with the number of previous preterm births.

Multiple Pregnancies

Around 40% of multiple pregnancies end in premature delivery. The implantation of more than one embryo during in vitro fertilization (IVF) increases the risk of a multiple pregnancy. With improved techniques there is increasing success with IVF using fewer embryos. Following a decision to transfer two instead of three embryos, the rate of twin birth in Sweden after IVF fell from 29% in 1991 to 18.5% in 2001, corresponding to a reduction in preterm birth of 72%. Improved techniques make it likely that a further reduction to a single embryo transfer will further reduce preterm deliveries, without any significant effect on the pregnancy rate (Källén et al. 2005).

Problems in Pregnancy

Elective Preterm Delivery

In approximately half of preterm births, rapid delivery is needed because of problems with the pregnancy. Maternal hypertensive disease is a common reason for elective preterm delivery, although this often is accompanied by other complications, particularly growth restriction. Advances in neonatal care and improvements in outcome for low-birth-weight infants have

resulted in an increase in the number of elective preterm deliveries, as obstetricians choose to deliver rather than risk continuing a pregnancy with a high risk of fetal loss, for example, severe intrauterine growth restriction.

Pregnancy-Induced Hypertension

Hypertension complicates 5% to 10% of all pregnancies and remains one of the major causes of maternal and neonatal mortality and morbidity. The commonest cause is preeclampsia (70% of cases) and is characterized by acute-onset hypertension, proteinuria, and edema. There may also be hemolysis, thrombocytopenia, and hepatic dysfunction as in the hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, which occurs in 2% to 12% of women with pregnancy-induced hypertension (PIH) (Geary 1997), and is associated with a high risk of maternal and perinatal mortality (Dotsch et al. 1997).

Preeclampsia is a systemic disease affecting the endothelium with resultant platelet activation and diffuse ischemic damage to all organs. The most obvious clinical manifestations involve the kidney (proteinuria, edema, and hyperuricemia), the liver (e.g., HELLP), and the brain (eclamptic convulsions). Ischemia caused by a defect of trophoblastic invasion of the spiral arteries (Khong et al. 1986) results in release of substances from the placenta that affect the endothelium, causing increased vascular reactivity and hypertension.

The only effective treatment is delivery and removal of the placenta, but optimal timing, when the baby is still significantly preterm, can be difficult. Antihypertensive drugs prevent cerebral hemorrhage in the mother but do not influence fetal outcome. Low-dose aspirin may prevent preeclampsia but only in those at high risk (Leitich et al. 1997). Use of aspirin in all pregnancies may be contraindicated because of a possible increased incidence of placental abruption (Sibai et al. 1993).

Can the development of preeclampsia be predicted? An increased resistance to blood flow in the uterine arteries during the second trimester has been shown in those at risk. This can be measured using Doppler ultrasound. In a group of mothers between 21 and 24 weeks' gestation, with evidence of increased resistance to flow, 58% developed PIH compared with 8% if measurements were normal (Zimmermann et al. 1997).

Intrauterine Growth Restriction

Failure of growth in an otherwise normal fetus is an important cause of elective preterm delivery. Intrauterine growth restriction (IUGR) is often associated with preeclampsia but it also occurs without maternal hypertension or proteinuria. The placenta, however, shows the same defect of placentation as found in preeclampsia and increased resistance indices in the Doppler waveform of the uterine arteries are predictive of poor outcome.

Decisions on Timing of Delivery in PIH and IUGR

Doppler ultrasound of flow in the umbilical artery and other fetal vessels can help in the difficult assessment of fetal well-being, and these techniques might reduce perinatal mortality (Neilson and Alfirevic 2002). In normal pregnancies the umbilical artery waveform shows a progressive increase in diastolic flow with advancing gestation (Erskine and Ritchie 1985), due to a fall in peripheral resistance secondary to continued growth and proliferation of the tertiary stem villi (Cohen-Overbeek et al. 1985). With abnormal placentation the peripheral resistance does not fall, and may even rise, resulting in a reduction in flow during diastole. In severe cases the diastolic flow is absent and can even show flow reversal. Absent end diastolic flow occurs only in abnormal pregnancies and is a serious sign of likely fetal compromise (Johnstone et al. 1988).

The compromised fetus will attempt to centralize its circulation to protect the brain and other vital organs. Increased blood flow in the middle cerebral artery, at the same time as reduced diastolic flow in the umbilical artery, is an indication of a severely compromised fetus.

Studies have clarified the order in which alterations in various vessel Doppler waveforms, fetal heart rate, and behavioral abnormalities take place, but they cannot indicate when delivery should take place in response to these changes (Ferrazi et al. 2002). The Growth Restriction Intervention Trial (GRIT) was a multicenter randomized controlled trial looking, in situations where there was uncertainty, at whether early delivery to

preempt intrauterine hypoxia improves outcome compared with delaying birth in an attempt to gain maturity. This was a large study, randomizing 548 women with evidence of fetal compromise at between 24 and 36 weeks' gestation. It showed a slight increase in stillbirths in the delayed delivery group but no difference in perinatal deaths (GRIT Study Group 2003). At 2 years of age there was a trend to more disability in the immediate delivery group (GRIT Study Group 2004). These results show that there is still no good way of detecting the optimum time for delivery in this high-risk group.

Maternal Disease

Diabetes

Insulin-dependent diabetes (type 1 diabetes) occurs in 3.5/1000 pregnancies and gestational diabetes affects a further 2% to 4%.

Diabetes results in increased risks of congenital malformations, abortion, preeclampsia, premature labor, late intrauterine death, fetal distress with obstructed labor, and birth asphyxia (see Chapter 24). Mortality rates and the incidence of congenital malformations remain high in the U.K. (Casson et al. 1997; Hawthorne et al. 1997), despite the fact that it is known that tight control reduces the risks to the fetus (Diabetes Control and Complications Trial Research Group 1996).

Poor control is associated with a higher risk of spontaneous preterm labor, and results in a macrosomic fetus with a high risk of neonatal complications such as hypoglycemia, respiratory distress, and jaundice.

Drug Abuse

Dependence on drugs, particularly cocaine, is a major risk factor for preterm delivery, but the pharmacological effects are difficult to disentangle from other deleterious factors associated with drug abuse: lack of antenatal care, use of other agents, poor nutrition, maternal depression, a stressful and disrupted social environment, and infection.

Rhesus Hemolytic Disease

Prophylaxis with anti-D has resulted in rhesus isoimmune disease becoming rare, but a small group of cases remains. Intervention is needed when there is evidence of ascites, a rising amniotic fluid bilirubin content, or fetal anemia. The treatment used depends on the severity of the disease, but fetal intravascular transfusion has proven successful in specialized centers, reducing the risk of intrauterine death and the need for preterm delivery of a sick infant. Antenatal anti-D prophylaxis should now be given routinely, at 28 and 34 weeks' gestation, to all nonsensitized women who are RhD negative.

Spontaneous Preterm Birth

Spontaneous labor may begin with uterine contractions or with premature rupture of membranes. The etiology is varied and in many cases no obvious cause is recorded, although with careful investigation a possible explanation can be found in the majority of cases (Lettieri et al. 1993).

Maternal genitourinary infection is a common association (Hillier et al. 1988). Lamont et al. (1986) reported abnormal colonization in 47% of preterm labors compared with 15% of controls and chorioamnionitis in 56% of the preterm group with only 10% in the controls. Vaginal carriage of *Gardnerella vaginalis* and *Ureaplasma urealyticum* result in significantly increased odds of spontaneous preterm birth (McDonald et al. 1992), although others have failed to show any association after adjustment for medical and sociodemographic factors (McGregor 1988). Coitus may contribute to ascending infection, and a definite relationship has been shown between sexual activity in the month before delivery and premature labor (Naeye 1980). Colonization of the genital tract, particularly with the genital mycoplasmas, is more common with young maternal age, primigravity, single marital status, black race, low income, low educational status, high number of sexual partners, smoking, and drug use—all factors that predispose to preterm delivery.

Infection can stimulate uterine contractions through prostaglandin synthesis. Antibiotics, therefore, might inhibit the progression to preterm delivery by interrupting the production of prostaglandins. However, the treatment of bacterial vaginosis has not been encouraging (Hauth et al. 1995; McDonald et al. 1997), although there may

be some benefit to the use of antibiotics if there has been a history of previous preterm delivery. Studies of antibiotics to prevent preterm birth use a wide range of different diagnostic methods, outcome parameters, and treatment schedules, which makes any systematic review difficult (Lamont 2005). Despite some promising results (Lamont et al. 2003; Ugwumadu et al. 2003; Kiss et al. 2004), the effect of prophylactic antibiotic treatment of bacterial vaginosis in early pregnancy has not been addressed with sufficient power in these studies.

For women presenting in preterm labor, the ORACLE trial randomized them to receive antibiotics or placebo. There was no difference in the primary outcome (a composite of death before discharge, oxygen at 36 weeks' gestational age, or major cerebral abnormality), between the groups (Kenyon SL et al., for the ORACLE Collaborative Group 2001a). However, in the subgroup with preterm prelabor rupture of the membranes, erythromycin given to the mother did reduce the primary outcome in singleton pregnancies (Kenyon SL et al., for the ORACLE Collaborative Group 2001b). In this same group co-amoxiclav was associated with some benefits but was also associated with an increased risk of necrotizing enterocolitis in the baby. This raises concerns about the effect of maternal antibiotics on the bacterial flora of the fetus and baby. However, there was no evidence of any effect on neonatal infection with gram negative or enterococci bacteria, although group B streptococcal bacteremia was significantly reduced (Gilbert et al. 2005).

Prolongation of pregnancy in the presence of infection may not be in the best interests of the fetus, with inflammatory cytokines being implicated in both the onset of preterm labor and white matter damage in the fetal brain (Hagberg et al. 2005). There is a link between chorioamnionitis and cerebral palsy. In the rabbit model, Yoon et al. (1998) have shown that experimentally induced chorioamnionitis treated with antibiotics and delayed delivery results in white matter lesions in the fetal brain.

Tocolytic Therapy

A number of therapeutic agents have been used to prevent progression of early spontaneous labor, but evaluation of these agents is complicated because preterm contractions do not always result in delivery. Of mothers entering trials of tocolytics, 50% remain undelivered after 1 week despite getting placebo (Anderson and Turnbull 1982).

Beta mimetics are widely used. They have a wide range of side effects, including pulmonary edema in 3% to 9% (Leveno and Cunningham 1992). Long-term therapy with the β-mimetic ritodrine has been associated with an increased risk of cerebral palsy (Takahashi et al. 1995). It is possible that this could be a consequence of prolonging fetal exposure to an adverse intrauterine environment.

Prostaglandin synthetase inhibitors are a logical choice because of the central role of prostaglandins in stimulating uterine contractions. Trials have proven their effectiveness, with fewer maternal side effects than the β- mimetics (Zuckerman et al. 1984). There are concerns about the safety for the fetus in whom constriction, and actual closure, of the ductus arteriosus has been documented (Huhta et al. 1987). There are no studies of sufficient power to allow exclusion of possible adverse effects in the baby following delivery. It has been associated with subsequent necrotizing enterocolitis (Major et al. 1994), bronchopulmonary dysplasia (Loe et al. 2005), and neonatal brain lesions, particularly periventricular leukomalacia (Baerts et al. 1990) and intraventricular hemorrhage (Doyle et al. 2005).

There is no direct evidence that tocolytics improve neonatal outcome. Their main purpose is to delay delivery to allow treatment with glucocorticoids and in utero transfer to a tertiary center, which may indirectly have beneficial effect on the immature central nervous system.

Reducing Morbidity and Mortality of Preterm Delivery

Steroids given to a mother in preterm labor improve pulmonary function in the baby, with increased surfactant synthesis and secretion, accelerated morphological development, and an increase in the number of pulmonary β-adrenergic receptors. A meta-analysis of 18 randomized trials showed a 50% reduction in incidence of respiratory distress in gestations up to 34 weeks (Crowley 1995). Neonatal death was reduced by

60%, and there were significant effects on necrotizing enterocolitis and intraventricular hemorrhage. Concerns about side effects, particularly the risk of infection in the presence of ruptured membranes, have not been supported by these studies. Corticosteroids are now recommended for all mothers in preterm labor between 24 and 34 weeks, although there may be benefit at earlier gestations. Betamethasone is preferred over dexamethasone, as the latter has not been shown to reduce the rate of neonatal death and has been associated with increased risk of periventricular leukomalacia, although these adverse effects may be due to the preservatives used in its preparation rather than the drug itself (Baud et al. 2001). Treatment is usually with 24 mg betamethasone as two doses 12 hours apart. Optimal benefit starts 24 hours after onset of treatment and lasts 7 days. There is no evidence to support multiple courses of corticosteroids, and animal and clinical data raise concerns about adverse effects on central nervous system structure and function, and fetal growth (Aghajafari et al. 1999).

Thyroid hormones also influence lung development, but, despite initial enthusiasm for the combined use of thyrotropin-releasing hormone (TRH) and steroids, a systematic review has shown no improvement in neonatal outcome (Crowther et al. 2004). Concerns have been expressed about maternal side effects (Crowther et al. 2004), while others have found TRH to be associated with long-term neurodevelopmental delay in the infant (Briet et al. 2002).

The Preterm Infant

Adaptation to Extrauterine Life

In the preterm infant the normal adaptive processes are complicated by a functional and structural immaturity of most systems.

Fluid Balance

Total body water decreases, from 86% of body weight at 24 weeks to 77% at term, due to a fall in extracellular water (ECW). After birth the contraction in ECW continues, resulting in the normal postnatal weight loss of around 10% of birth weight in the term infant. As the preterm infant has proportionally more ECW, the weight loss is often greater, up to 15% of birth weight. Renal function in the preterm baby is immature, but the kidney has the capacity to concentrate and dilute the urine, although there is a limited ability to excrete an excessive water load, and problems of fluid balance are common. A delay in the postnatal reduction in ECW has been associated with more severe lung disease, patent ductus arteriosus, intraventricular hemorrhage, and bronchopulmonary dysplasia (Modi 1993).

While term infants have a cornified layer in the epidermis, the preterm infant has a poorly developed epidermal layer, only two to three cells thick, and no efficient barrier to diffusion of water resulting in excessive transepidermal water loss (TEWL). This is dependent on gestation, postnatal age, and environmental factors such as humidity and temperature, with TEWL decreasing over the first 2 weeks of life as the barrier function of the epidermis improves (Hammarlund et al. 1983).

Temperature Control

The evaporation of water from the skin is also the most significant cause of heat loss in preterm infants. Allowing the preterm baby to get cold immediately after birth has been associated with increased mortality (Hazan et al. 1991; Costeloe et al. 2000). All thermoregulatory mechanisms are immature; in particular, there are low stocks of brown fat, and thermal control is a major problem in the first weeks of life. Temperature instability adds to the morbidity of these babies at a time when they have maximum effects from other complications of preterm birth.

Glucose Metabolism

With removal of the placenta, blood sugar falls following delivery, and glycogen is the primary source of glucose in the immediate newborn period. The preterm baby, with limited glycogen stores, is at increased risk of hypoglycemia. When compared with adults, the brain of the newborn appears to be more resistant to hypoglycemia, suggesting it is able to use substrates other than glucose. The brain of newborn animals can utilize lactate and ketones, but there is no direct evidence for this in humans, and the preterm infant has

a limited ability to mobilize alternative fuels (Hawdon et al. 1992).

The apparent resistance to hypoglycemia has led to the acceptance of lower glucose levels in the newborn baby and to the belief that asymptomatic hypoglycemia is of little consequence. However, blood sugars below 2.5 mmol/L, even when asymptomatic, have been associated with poorer neurodevelopmental outcomes (Lucas et al. 1988) and with neurophysiological disturbances (Koh et al. 1988). Current recommendations are that the blood glucose in the newborn, whatever the gestation, should be maintained above 2.5 mmol/L.

Respiratory Problems

Respiratory Distress Syndrome

Respiratory distress syndrome (RDS), previously known as hyaline membrane disease, is a common problem in the preterm infant presenting with tachypnea, intercostal and subcostal recession, a grunting noise in expiration, and increasing oxygen requirements developing soon after birth. Chest x-ray shows atelectasis with fine reticular-granular infiltrates and an air bronchogram (Fig. 11.1). In severe cases the x-ray appearances progress to bilateral opacity—the "white out." Radiographic patterns, however, are variable and do not necessarily correlate with the degree of respiratory compromise.

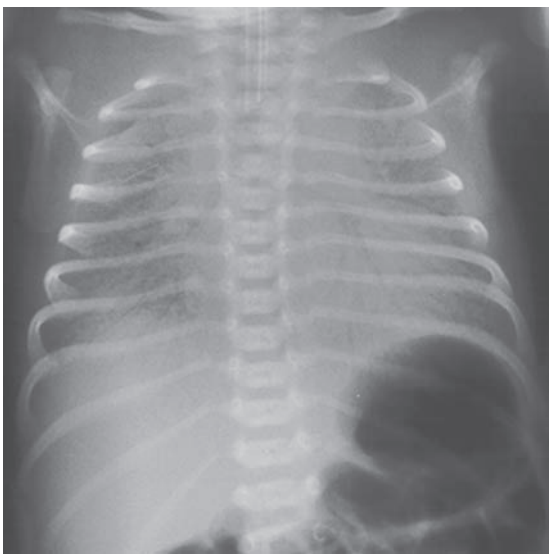

Figure 11.1. Chest x-ray showing respiratory distress syndrome.

The infant attempts to maintain alveolar volume by prolonging and increasing expiratory pressures by breathing against a partially closed glottis, causing the typical grunting noise. In severe cases there is a rapid increase in oxygen requirement and progression to ventilation in the first 24 hours. In uncomplicated cases recovery occurs within a week with no further oxygen requirements. The incidence and severity of RDS increase with decreasing gestational age and male sex. There is also an increased risk in infants of diabetic mothers or multiple births, and in infants with fetal asphyxia.

Pulmonary surfactant is found in increasing amounts with rising gestation, and qualitative and quantitative abnormalities of this substance are critical to the pathogenesis of RDS. Surfactant gives stability to alveoli, by reducing surface tension, preventing their collapsing at end expiration, and it is also important in maintaining patency of conducting airways as well as in helping match alveolar capillary filling with ventilation. The function of surfactant can be reduced by a number of inhibitors, particularly plasma proteins found in the lungs in diseases such as RDS.

The surfactant lipids, of which dipalmitoylphosphatidylcholine (DPPC) is the most abundant, form a monolayer at the air–liquid interface of the alveolar surface. The active material also contains 5% to 10% of surfactant proteins (SP)-A, -B, -C, and -D. Hydrophobic SP-B and SP-C are crucial in catalyzing the insertion of surfactant lipids into the surface film. Their functional importance is seen in the rare familial condition of SP-B deficiency, which results in severe respiratory dysfunction from birth. The functions of the hydrophilic SP-A and SP-D are less certain but they play a part in the defense mechanism of the lung. SP-A has a protective effect for surfactant against the inhibitory effects of plasma proteins.

Replacement surfactant can be given by bolus intratracheal injection via an endotracheal tube and has been shown to reduce mortality by 40% to 50% and pulmonary air leaks by 30% to 70% (Halliday 1996). Animal-derived surfactants are currently the treatment of choice, as they have

been shown to significantly reduce mortality in neonates when compared with protein-free synthetic drugs (Soll and Blanco 2003). A new generation of synthetic surfactants is being developed and evaluated.

Prophylactic treatment, defined by some as "administration in the delivery room" and by others as "surfactant given before start of ventilation," has been shown to be associated with a better outcome compared with the use of surfactant as rescue therapy in established disease (Soll and Morley 2003). Many babies do not require a further dose of surfactant. Traditionally a second dose was always given after 12 hours but it seems more appropriate to tailor the regime to the infant's disease status.

Pulmonary Air Leaks

The preterm baby is at risk from pulmonary air leaks, usually as a complication of ventilatory support for lung disease. High transpulmonary pressure swings result in alveolar overdistention and rupture. The gas tracks alongside the vessels to the mediastinum and from there may rupture into the pleura, pericardium, or extrathoracic areas. Some gas gets trapped in the parenchyma of the lung, resulting in pulmonary interstitial emphysema.

In Edinburgh the incidence of pneumothorax in ventilated babies less than 32 weeks' gestation has fallen steadily from 15% in 1993 to only the occasional baby in the last 5 years. Contributing to this improvement is the increased use of antenatal steroids, prophylactic surfactant replacement therapy, and better control and monitoring of ventilation aimed at reducing injury by preventing overdistention of the lung.

Pneumothorax causes a deterioration in the clinical condition and is associated with a high risk of intraventricular hemorrhage (Lipscombe et al. 1981). There is a significant mortality, with rates as high as 53% in babies less than 1000 g birth weight (Greenough and Roberton 1985).

Pulmonary interstitial emphysema (PIE), gas trapped within the perivascular sheaths of the lung, reduces pulmonary perfusion and interferes with ventilation (see Figs. 17.9 and 17.10 in Chapter 17). Mild degrees often disappear if further injury to the lung is prevented. In severe cases various maneuvers have been tried including selective intubation of one main bronchus and creation of a pneumothorax to allow the interstitial air to drain (Milligan et al. 1984). Mortality is between 24% and 50%, depending on severity, and bronchopulmonary dysplasia is common in survivors.

Pneumopericardium is usually associated with other air leaks and may be asymptomatic but often causes cardiac tamponade with hypotension, bradycardia, and cyanosis (see Fig. 2.9 in Chapter 2). The chest x-ray is diagnostic, with air outlining the heart. Drainage can be achieved with improvement in the baby's condition but mortality is high at 80% to 90%.

Systemic air embolism is a rare complication of ventilation and results in a sudden and catastrophic deterioration in the baby's condition, leading inevitably to death. An x-ray done just after death will show bubbles of gas in the systemic and pulmonary arteries and veins. It results from alveolar–capillary or bronchovenous fistulas, which have been demonstrated by barium studies at autopsy (Bowen et al. 1973).

Chronic Lung Disease (Bronchopulmonary Dysplasia)

Northway et al. (1967) coined the term *bronchopulmonary dysplasia* (BPD) for the chronic lung disease (CLD) occurring in infants recovering from respiratory distress syndrome, or other lung problems, after prolonged exposure to high levels of ventilatory support and oxygen. The condition described was characterized by interstitial and alveolar edema early in the clinical course and subsequently with persistent inflammation, fibrosis and small airway disease. On x-ray the lungs showed overinflation with a combination of cystic emphysema and fibrosis (Fig. 11.2). This picture is less common today and is seen mainly in mature babies who survive prolonged ventilation for severe respiratory problems such as meconium aspiration or diaphragmatic hernia. Most babies who now develop CLD have birth weights below 1000 g. The x-rays appear uniformly hazy with progression to a fine lacy parenchymal pattern with modest hyperinflation and few large cysts (Toce et al. 1984) (Fig. 11.3). Compared with traditional BPD, the lung anatomy in these immature

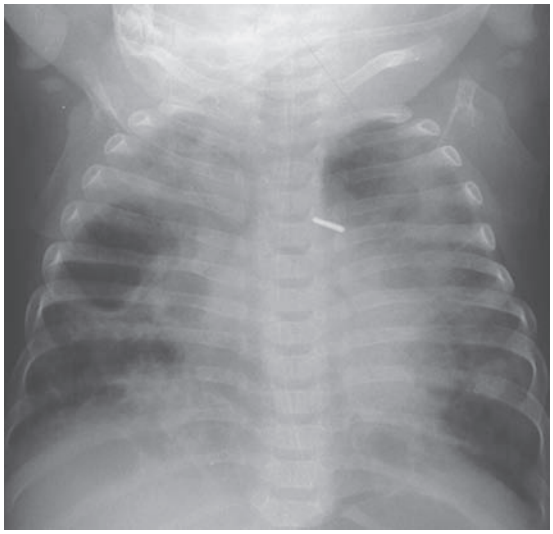

Figure 11.2. Chest x-ray of bronchopulmonary dysplasia. Note surgical clip on ductus arteriosus.

infants shows minimal small airway injury but dilated distal gas exchange structures, decreased alveolarization, and less prominent inflammation and fibrosis (Erickson et al. 1987) (the pathological features of respiratory disorders associated with prematurity are described in Chapter 20). Relative to age-matched controls, preterm baboons that survived for 33 weeks after ventilation for the initial 2 weeks of life had 52% fewer alveoli and alveolar surface area was decreased by 33% (Coalson et al. 1995). Jobe and Ikegami (1998) review the mechanisms initiating lung injury and postulate that the "new" BPD is not primarily the injury/repair paradigm of the "old" BPD but rather a maldevelopment sequence resulting from interference/interruption of normal developmental signaling for terminal maturation and alveolarization of the lungs of very preterm infants. The maximum rate of accretion of alveoli is over a period from about 25 weeks' gestation to around 4 months after birth.

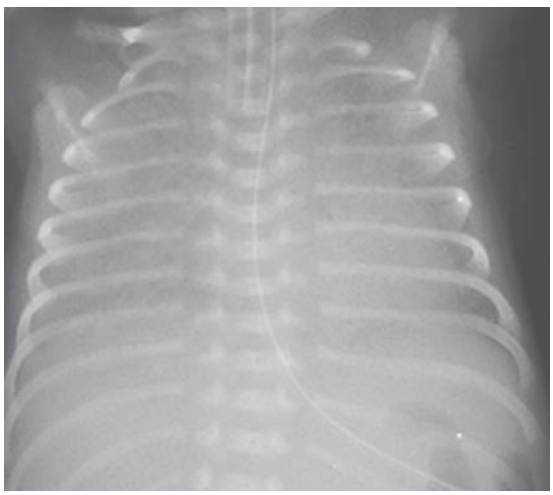

Figure 11.3. Chest x-ray of the new bronchopulmonary dysplasia (BPD).

Chronic lung disease is associated with a significant inflammatory response in the lungs (Groneck and Speer 1995) and high levels of inflammatory cytokines, including interleukin-1 (IL-1) and interleukin-8 (IL-8), are found in the alveolar secretions (Groneck et al. 1994). There is increasing evidence that antenatal exposure to proinflammatory cytokines, as found for example in chorioamnionitis, can predispose to the development of BPD, independently of the severity of RDS (Watterberg et al. 1996). Ventilation from birth is associated with a significant rise in proinflammatory mediators within the airways (Groneck and Speer 1995). Neutrophils and macrophages attracted to the site of injury can cause severe lung damage by release of proteases and by generation of toxic oxygen radicals that are poorly dealt with by the immature antioxidant activity in the preterm infant. However, proinflammatory mediators also interfere with the presently unknown signaling pathways that are important to lung maturation and alveolarization, and it is postulated that this may be the final common pathway for the development of CLD in the immature infant in whom alveolarization is just beginning and is most susceptible to interference (Jobe and Ikegami 1998).

Rojas et al. (1995) has noted that the major association, other than birth weight and gestational age, with CLD for infants without significant lung disease at birth is postnatal sepsis. The incidence of ascending infection is higher at early gestations and may be a major cause of preterm labor (Watts et al. 1992). There is an association between infection at birth with *Ureaplasma urealyticum* and the development of CLD, but a

cause-and-effect relationship has not been proven (Cassell et al. 1988). These organisms have been shown to induce an inflammatory bronchopulmonary response (Groneck et al. 1996) and have been associated with a specific clinical and radiological picture in the preterm infant (Theilen et al. 2004). However, eradication of the genital mycoplasma by the use of erythromycin from birth did not produce any beneficial effect (Lyon et al. 1997), although further intervention studies are needed.

Chronic lung disease is commonly defined as a persistent oxygen requirement at 28 days of age in a baby who was ventilated after birth. This is, however, a poor predictor of long-term outcome, and a better prognostic indicator is to use a definition of oxygen dependence at a postconceptional age of 36 weeks (Shennan et al. 1988). In Edinburgh over the last 10 years the incidence of CLD, using the latter definition, has remained at around 50% of all surviving infants below 30 weeks' gestation.

Presentation is with delayed resolution of RDS, persistent need for supplemental oxygen, and frequently respiratory support, either with positive-pressure ventilation or nasal continuous positive airway pressure (CPAP) after 1 month of age. Feeding difficulties and aspiration are common due to associated bulbar dysfunction and gastro-esophageal reflux. These problems exacerbate the poor growth of many of these infants who require an increased calorie intake to deal with the high metabolic demands of this condition. Cor pulmonale is a late complication, particularly if the baby is chronically hypoxic. The skeleton calcifies poorly, and osteopenia is common, resulting in a very compliant chest wall, which can contribute to the persistent respiratory problem (Glasgow and Thomas 1977).

Chronic lung disease is an important condition that, with improved survival of the very preterm infant, contributes an increasing workload to neonatal units. Antenatal steroids and replacement surfactant, particularly if given early (Stevens et al. 2004), have reduced the ventilator and oxygen requirements of these babies but have not influenced the incidence or severity of CLD (Hoekstra et al. 1991), further evidence that even mild insults have a significant effect on subsequent lung development. Ventilator strategies are aimed at reducing ongoing lung injury, but no particular mode of ventilation has been shown to lower the incidence of CLD. High-frequency oscillation showed early promise but a large randomised study did not show any benefit over conventional ventilation (Johnson et al. 2002).

Once CLD is established, ventilator management is aimed at reducing further lung trauma. Infection, including viral infection, is a common cause of deterioration. Diuretics have a role particularly in those who are fluid overloaded, but there is no evidence that they improve long-term outcome and they cause problems with electrolyte imbalance and calciuria, leading to nephrocalcinosis (Hufnagle et al. 1982).

Steroids have been used successfully to wean babies from ventilation (Cummings et al. 1989), but there is little evidence that they affect long-term outcome. There are concerns about side effects of steroids (Ng 1993). Hypertension is common (Greenough et al. 1992), and these drugs exert a marked catabolic effect at a critical time in the baby's growth (Brownlee et al. 1992). A reduction in head growth has been shown, with no catch up after therapy ends (Shrivastava et al. 2000). This implies a disturbance in brain growth, and is in keeping with the known effects of steroids on glial proliferation (Howard and Benjamins 1975). Of most concern is the reported adverse effects on long-term neurodevelopmental outcome, with one additional case of cerebral palsy being reported for every 17 babies treated (Halliday et al. 2003). These findings have resulted in a marked reduction in the use of postnatal steroids in the preterm baby, particularly in the first weeks of life. Later use of dexamethasone does not appear to be associated with the same risk of cerebral palsy. Inhaled steroids have not been shown to be effective.

Despite improvement in lung function, there is also evidence that postnatal lung development may be impaired by the use of steroids, given either antenatally (Ikegami et al. 1997) or after birth (Tschanz et al. 1995), with the treated lung showing a reduction in the formation of its full complement of alveoli.

More work is needed to determine the optimum dose and timing of postnatal steroids, but any studies must include long-term follow-up.

Patent Ductus Arteriosus

In the absence of respiratory distress syndrome (RDS) the ductus arteriosus closes in the same time frame after birth in preterm as it does in term infants, with over 90% being closed by 60 hours of age. Prematurity and RDS are the two major factors related to persistence of the ductus arteriosus.

Premature infants with a patent ductus arteriosus (PDA) are at increased risk of more severe and prolonged RDS, chronic lung disease, and death. Prophylactic indomethacin reduces the need for surgical ligation of the duct and is associated with a reduction in the rate of severe intraventricular hemorrhage, without any significant adverse effects. However, there is no evidence that indomethacin used this way affects important long-term outcomes, such as death or severe neurosensory impairment (Fowlie and Davis 2004). Prophylactic ibuprofen has not been shown to be superior to indomethacin (Van Overmeire et al. 2004). It has no effect on intraventricular hemorrhage, and one study was stopped early because of the development of pulmonary hypertension in the treated group (Gournay et al. 2004).

Many clinicians treat the ductus arteriosus only if it is thought to be causing a significant hemodynamic problem. There is no agreement as to the optimum timing and method for treating a PDA (Fowlie 2005). Most would consider therapy with drugs or surgery if the baby is not weaning from ventilation and there is clinical and echocardiographic evidence of a significant left to right shunt.

Brain Injury in the Preterm Infant

Of infants born with a birth weight below 1500 g, approximately 85% survive and of these 5% to 15% develop spastic motor defects. Another 25% to 50% show cognitive/behavioral deficits of varying degrees that result in learning difficulties and school failure.

The major neuropathologies in the preterm infant are periventricular hemorrhagic infarction, a hemorrhagic necrosis of the periventricular white matter usually associated with intraventricular hemorrhages, and periventricular leukomalacia.

Intraventricular Hemorrhage

Hemorrhage into the germinal matrix with spread of blood to the lateral ventricles is the commonest form of intracerebral bleed seen in the preterm baby. Papile et al. (1978) reported intraventricular hemorrhage (IVH) in 43% of infants with a birth weight below 1500 g, but through the 1980s this decreased to 20% to 25%, mainly due to the increased use of antenatal steroids along with advances in neonatal care (Maher et al. 1994; Rennie et al. 1996).

The occurrence of a primary germinal matrix hemorrhage is rare after the first 72 hours of life, but an existing bleed can still extend beyond that time. The clinical features can be mild or nonexistent, but some infants develop a worsening of their respiratory disease along with cardiovascular instability, particularly a marked variability in blood pressure, and an unexpected drop in hemoglobin. Diagnosis is made using cranial ultrasound, and IVH has been graded depending on the extent of the bleed (Table 11.3).

Grade 4 hemorrhage (Fig. 11.4) occurs in 10% to 15% of cases, and the likely mechanism is one of venous infarction caused by a reduction in perfusion of the white matter adjacent to the ventricle, resulting in periventricular hemorrhagic infarction. This is usually asymmetric in size, with 67% being unilateral. Hemorrhage may also occur into areas of the brain that were previously ischemic (Rushton et al. 1985). This hemorrhagic periventricular leukomalacia occurs in approximately 15% of all cases of periventricular leukomalacia.

The main predisposing factors are prematurity and respiratory distress syndrome, with IVH occurring because of a combination of hemodynamic instability and an intrinsic bleeding tendency. Rapid alterations in systemic blood pressure occur with many procedures, for

Table 11.3. Grading of intraventricular hemorrhage (Papile et al., 1978)

Grade 1	Bleed confined to the germinal matrix
Grade 2	Blood in the lateral ventricle but with no ventricular dilation
Grade 3	Blood in lateral ventricle with ventricular dilation
Grade 4	Parenchymal involvement

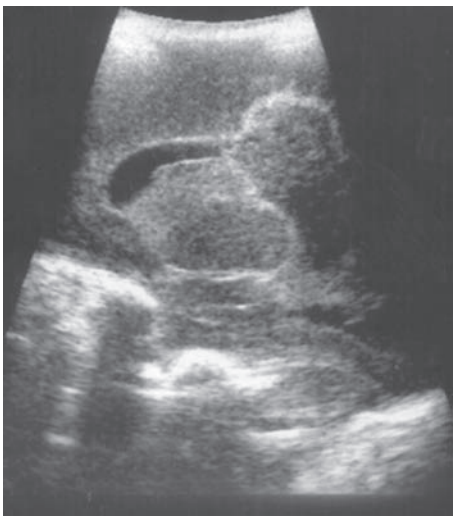

Figure 11.4. Parasagittal ultrasound scan showing parenchymal extension of intraventricular hemorrhage.

example, handling, endotracheal tube suction, and ventilation, and, because of disturbed cerebral autoregulation in the preterm baby, these fluctuations are transmitted directly to the immature, poorly supported germinal matrix vasculature. The risk of bleeding is further increased if factors causing endothelial damage are present, such as asphyxia, acidosis, and hypertension. Preterm infants have low levels of several clotting factors, and the neonatal platelet has a storage pool defect.

The risk of IVH is reduced with the use of antenatal steroids and with good intensive care in which blood pressure and gases are kept stable. None of the various agents used for prophylaxis, including indomethacin, ethamsylate, and vitamin E, have gained general acceptance.

Ventricular dilatation is a direct consequence of IVH. In some this will resolve with time but others are left with persistent dilatation, and in a small number this is progressive with the development of posthemorrhagic hydrocephalus.

Ischemic Lesions to the Brain—Periventricular Leukomalacia

Banker and Larroche (1962) introduced the term *periventricular leukomalacia* (PVL) in their description of 51 cases. Many of the etiological factors of this condition are in keeping with the observation of Takashima and Tanaka (1978) that the lesions occur in the vascular watershed areas of the brain.

Periventricular leukomalacia can develop several weeks after birth and is seen in 2% of very low birth weight survivors. On ultrasound, areas of increased echodensity appear within 24 to 48 hours of the insult, with cysts evolving 2 to 4 weeks later (Trounce et al. 1986) (Fig. 11.5). The cysts remain visible for several weeks but eventually disappear, leaving glial scars or generalized cerebral atrophy.

Prematurity is not such a clear risk factor for PVL as it is for IVH (Ikonen et al. 1988), but many of the known associations are more commonly seen in preterm babies. The major etiological factors relate to ischemic insults to the brain, to which the actively differentiating periventricular glial cells are particularly vulnerable, and to circulating inflammatory cytokines. Chorioamnionitis is associated with both cerebral palsy and PVL in preterm infants (Wu 2002). Culture positive infections remote from the brain have also been shown to increase the risk of white matter damage in the preterm brain (Dammann and Leviton 1998; Graham et al. 2004; Dammann et al. 2005).

Cerebral blood flow is directly dependent on systemic blood pressure, and PVL is closely associated with episodes of hypotension and with cerebral vasoconstriction caused by hypocarbia from overventilation.

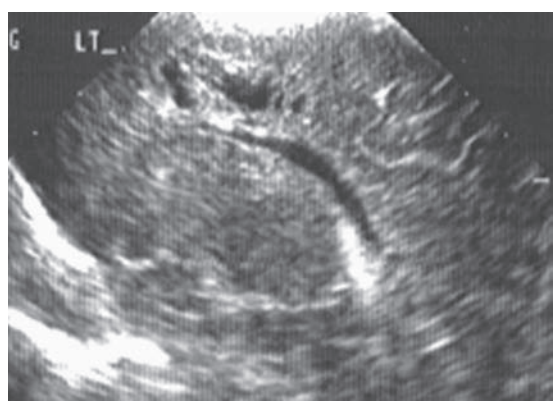

Figure 11.5. Cystic change in periventricular leukomalacia.

Prognosis

A normal cerebral ultrasound scan is associated with a 90% chance of a normal outcome. Intraventricular hemorrhage alone has a similarly good prognosis unless complicated by persistent ventricular enlargement, where the chance of a normal outcome is about 60%, reducing to 25% with shunted hydrocephalus. White matter damage is always significant, and cystic PVL is the most powerful predictor of cerebral palsy (Pinto-Martin et al. 1995), with 90% of the cases being associated with major handicap. Later magnetic resonance imaging has shown delayed myelination, suggesting that the cysts are markers of even more diffuse injury to oligodendroglia (de Vries et al. 1993). There is a fivefold increase in the risk of cerebral palsy following ultrasound diagnosis of any parenchymal lesion and a 15-fold increase in the presence of bilateral occipital periventricular leukomalacia (Rennie 1997). Learning difficulties may in some cases be due to lesser degrees of white matter damage, but prediction of these problems remains difficult (Levene et al. 1992), although visual handicap can be accurately predicted from ultrasound abnormalities (Pike et al. 1994).

Nutrition of the Preterm Infant

Breast Milk

Human milk does not meet the theoretical requirements of the preterm infant because of insufficient protein, energy, electrolytes, and minerals. However, fresh breast milk is well tolerated and is protective against infection and necrotizing enterocolitis as well as being important in stimulating gut function. Developmental outcome at 18 months has been shown to be better in preterm babies fed human milk (Lucas et al. 1990), and it is interesting to speculate if this advantage may be related to the type of fats in milk.

Fat is not just a source of energy but is composed of a series of complex hydrocarbon structures necessary for the formation of membranes. Of particular importance are polyunsaturated fatty acids (PUFAs), particularly docosahexanoic and arachidonic acid, found in high concentrations around the synapses. The preterm baby is unable to synthesize these acids from the essential fatty acids—linoleic and linolenic acid. Human milk however supplies docosahexanoic and arachidonic acids, and breast-fed babies have significantly higher concentrations of them in their cerebral cortex compared with those fed formula. The consequences of this are unknown, but preterm infants fed formula have significantly different electroretinographic patterns, indicating delayed rod photoreceptor maturation, than those fed human milk or given supplementary docosahexanoic acid. The supplemented group also performed significantly better than controls on developmental testing (Cockburn 1994). Milk companies now supplement their preterm infant formulas with PUFAs.

Problems of Parenteral Nutrition

Many sick newborns cannot tolerate sufficient amounts of milk, and intravenous amino acid and fat solutions are a common part of their early management. The central venous catheters used for parenteral nutrition become infected, often with coagulase-negative staphylococci, in at least 20% of cases, and can also cause superior vena cava (SVC) or inferior vena cava (IVC) obstruction, cardiac arrhythmias, pleural and pericardial effusions, or chylothorax. The infants have abnormal amino acid profiles, although the significance of this is unknown, and there is concern about the deposition of fat in the lungs as a possible etiological factor in chronic lung disease (Cooke 1991). Cholestatic jaundice occurs in 10% to 40% possibly as a result of immaturity of the hepatobiliary system, prolonged fasting, coexisting sepsis, and impaired bile secretion. In most cases cholestasis resolves when enteral feeding starts, but progression to biliary cirrhosis and liver failure can occur.

Bone Disease

Metabolic bone disease is common in the preterm baby, and its features include radiological abnormalities such as osteopenia, rachitic changes, and fractures. Raised alkaline phosphatase and hypophosphatemia are common biochemical findings. The pathology shows generalized skeletal demineralization and a disordered metaphyseal

cartilage plate similar to that seen in classic rickets (Oppenheimer and Snodgrass 1980). However, these babies have elevated levels of the vitamin D metabolites, particularly 1,25-dihydroxycholecalciferol (Markestad et al. 1984).

Phosphorus deficiency is associated with metabolic bone disease, and hypophosphatemia is seen commonly in preterm neonates fed human milk and during parenteral nutrition. Bone mineral accretion rates similar to those seen in utero can be achieved by increasing mineral intakes (Steichen et al. 1980), but without dietary enhancement bone mineral accretion is negligible (Horsman et al. 1989).

Severe bone disease results in fractures and has been associated with increasing respiratory disease as a consequence of a soft compliant chest wall (Glasgow and Thomas 1977). The lesions, including fractures, usually heal spontaneously, and there is no evidence that these babies have more fragile bones in later childhood. However, a high plasma alkaline phosphatase activity has been associated with reduced body length at 18 months (Lucas et al. 1989).

Necrotizing Enterocolitis

This overwhelming gastrointestinal illness affects 5% to 30% of babies born weighing <1500 g. The prevalence ranges from 4% to 22% (Uauy et al. 1991), and the suggestion from this study was that the risk of necrotizing enterocolitis (NEC) is affected by clinical practice; that is, NEC has an iatrogenic component.

Over 90% of those developing NEC are born at less than 34 weeks' gestation and most have been enterally fed (Stoll 1994). Of the risk factors associated with NEC (Table 11.4), prematurity and enteral feeding are the only two that are firmly established.

Table 11.4. Risk factors for necrotizing enterocolitis

Prematurity	Hypothermia
Intrauterine growth retardation	Patent ductus arteriosus
Abruptio placenta	Cyanotic heart disease
Prolonged rupture of membranes	Polycythemia
Asphyxia	Anemia
Low Apgar scores	Non–human milk formula
Umbilical catheterization	Hypertonic feeds
Exchange transfusion	"Rapid" enteral feeding
Hypoxia and shock	Fluid overload
	"Necrogenic" bacteria

In term infants NEC occurs soon after birth and in babies who have not been fed, and the likely initiating event is an ischemic insult to the gut, often following birth asphyxia. Onset in the preterm infant is usually after the first week of life and is associated with enteral feeding, but not with perinatal asphyxia, RDS, or the status of the systemic circulation, including patent ductus arteriosus (Kanto et al. 1987). Umbilical artery catheterization increases the risk sevenfold, but there is no difference whether the catheter is placed above the diaphragm or below the origin of the renal and mesenteric arteries (Clark et al. 1993). Preterm babies who are also growth restricted, and in whom there has been absent or reverse end diastolic flow in the fetal umbilical vessels, have been shown to be at increased risk of NEC (McDonnell et al. 1994), due possibly to an ischemic insult to the gut caused by centralization of the circulation in the compromised fetus. Others have failed to confirm this association (Adiotomre et al. 1997). Tocolysis with indomethacin has been associated with NEC in low-birthweight infants (Major et al. 1994), but there is no association between the use of indomethacin postnatally, in the management of patent ductus arteriosus, and NEC (Bauer 1992). Overall, there is no convincing evidence in the preterm infant for a relationship between ischemia–reperfusion injury and NEC.

Approximately 5% of cases of NEC occur in clusters, suggesting an infectious component, but no single organism has been implicated as the cause.

Enteral feeding is the major risk factor for NEC in the preterm infant, and it is likely that the initiating event is intestinal mucosal disruption. Small intestinal motor activity and colonic function are immature, resulting in delayed intestinal transit, which permits bacterial overgrowth and impairs clearance of products of fermentation. There is evidence of a link between carbohydrate intolerance and NEC (Clark and Miller 1990), and accumulation of organic acids from fermentation of undigested carbohydrates can cause mucosal damage. Preterm babies are at greater risk of NEC if they have been formula fed, and the volume of feeds is increased. Breast milk is protective

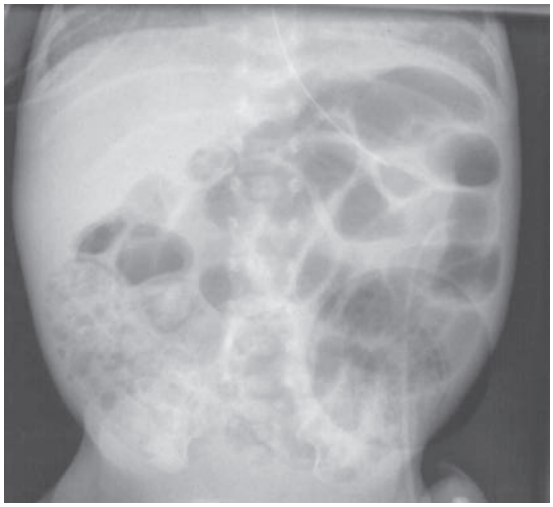

Figure 11.6. Necrotizing enterocolitis with pneumatosis intestinalis.

possibly due to epidermal growth factor and prostaglandins, which enhance mucosal development, as well as secretory immunoglobulin A (IgA) and lactoferrin, which modulate gut flora.

The classic presentation is a rapid onset of abdominal distention, bloody mucousy stools, and bilious aspirates or vomits. It can also present with nonspecific symptoms such as lethargy, unstable temperature, and apneic attacks that slowly progress to the signs of severe sepsis.

The pathognomonic radiological sign in NEC is bubbles of intramural gas (pneumatosis intestinalis) (Fig. 11.6), although in many cases the x-ray appearance may be nonspecific with dilated loops of gut, thickening of the bowel wall, and fluid levels. Gas is sometimes seen in the biliary tree. A fixed dilated loop is suggestive of gangrene of the bowel. Perforation occurs in about one third of cases.

Treatment involves stopping enteral feeds, giving antibiotics, and providing supportive care including parenteral nutrition. Surgery is indicated if there is a perforation or evidence of gangrenous bowel, which can often be diagnosed using abdominal paracentesis.

Reported mortality rates of 20% to 49% are directly related to bacteremia, intravascular coagulopathy, ascites, and very low birth weight, all of which are poor prognostic features. Later complications are mainly due to stricture formation presenting within 3 months in 10% to 20% of cases. The most common sites are in the transverse or descending colon. Extensive surgical resection of the gut can result in serious problems due to "short gut." These babies may need long-term parenteral nutrition, and some may require liver and small bowel transplants.

Antenatal steroids have been shown to reduce the risk of NEC, presumably due to an enhancement of the mucosal barrier. Breast milk is protective but there is no evidence that other treatments, particularly prophylactic antibiotics or immunoglobulin, affect the incidence of NEC.

Retinopathy of Prematurity

Retinopathy of prematurity (ROP) was first described in the 1940s and termed retrolental fibroplasia (Terry 1942). The incidence dropped in the 1950s, when it became clear that uncontrolled high concentrations of oxygen were important in its etiology, but has risen again as a result of the improved survival of very preterm infants. It is the cause of blindness in 8% of extremely low birth weight infants (Doyle 1995).

Retinopathy of prematurity is a condition of the immature retinal vasculature occurring at the junction of the vascularized and yet to be vascularized retina by the production of angiogenic factors leading to vasoproliferation. The stimulus to production of these factors is unknown.

The condition is staged according to its appearance (Table 11.5). Treatment with laser coagulation or cryotherapy is needed for extensive stage 3 changes, particularly if there is also evidence of rapidly advancing disease with tortuous and engorged retinal vessels, often with a rigid and

Table 11.5. Staging of retinopathy of prematurity

Stage	Description
1	Demarcation line, lying in plane of retina, at junction of vascularized and avascular retina
2	Ridge; the demarcation line extends out of the plane of the retina
3	Ridge with extraretinal fibrovascular proliferation; neovascularization may extend into the vitreous
4	Subtotal retinal detachment
5	Total retinal detachment

unreactive iris—the so-called "plus disease." In addition the presence of plus disease suggests rapidly advancing disease. In this, the iris is often rigid and unreactive and the retinal vessels appear tortuous and engorged.

Retinopathy of prematurity occurs in 30% to 60% of babies of birth weight <1500 g, and the incidence and severity rise with decreasing gestational age. Severe disease (stage 3 or above) is virtually confined to those infants of birth weight <1500 g and <31 weeks gestational age. In Edinburgh in the last 5 years, all cases with grade 3 ROP were less than 29 weeks' gestation, and 70% of these babies required treatment with laser therapy.

The timing of onset is more closely related to postmenstrual than to postnatal age, with acute ROP developing between 32 and 44 weeks. It is rare before 31 weeks and disease commencing after 36 weeks is unlikely to become severe.

Of the risk factors, prematurity is the most powerful. Oxygen is important, but it may be fluctuating levels that are significant rather than sustained high levels (Cunningham et al. 1995). Blood transfusions, apneic episodes, hypercarbia, acidosis, intraventricular hemorrhage, and vitamin E deficiency have all been implicated, as has early exposure to light (Lucey and Dangman 1984). Replacement surfactant has increased survival of the very low birth weight infant, but its effect on ROP is not proven, although some data suggest that prophylactic surfactant may reduce the severity of severe ROP (Pennefather et al. 1996).

All babies ⩽1500 g or <32 weeks are now screened, starting 6 to 7 weeks after birth, with repeated examinations until the retina is fully vascularized. The current guidelines on screening and treatment are an effective and efficient means of detecting treatable ROP (Fleck et al. 1995), and in the U.K. most babies are being screened according to the protocol (Haines et al. 2005). Retinal detachment and blindness can still occur after screening and treatment, but this should now be a very rare event.

Infection

Sepsis and meningitis are important causes of morbidity and mortality in the newborn baby, and the onset occurs within the first 10 days of life in 90% of the cases in preterm infants. In a large cohort study, neonatal infection among extremely low birth weight infants has been associated with poor neurodevelopmental and growth outcomes in early childhood (Stoll et al. 2004).

Low birthweight is significantly associated with bacterial sepsis and meningitis; the smaller the infant, the higher the incidence of infection (Vesikari et al. 1985). The rates in infants of birth weight 1000 to 1499 g are twice those of 1500 to 2000 g and eight times those above 2000 g.

Infants in intensive care units have a high rate of sepsis (Simon et al. 1991), which is an important factor in late mortality. In these babies the risks are increased by invasive procedures and indwelling catheters, particularly central venous lines.

A wide variety of bacteria may produce infection. *Escherichia coli* remains a constant threat throughout the neonatal period, with a high fatality rate. In the first few days of life, group B streptococcus is most prominent, but later in the neonatal period the most common pathogen is coagulase-negative staphylococcus, usually *Staphylococcus epidermidis*, although *Staphylococcus aureus* is also frequently seen. Coagulase-negative staphylococcal infection is currently responsible for most late-onset neonatal sepsis. Although this organism can cause serious disease, most infections are relatively benign: meningitis is rare and mortality low compared with infection from other organisms (Isaacs, on behalf of the Australasian Study Group for Neonatal Infections 2003).

The preterm baby is often colonized with *Candida albicans* from birth and it can be isolated from the mouth and perineum. Disseminated candidiasis is rare in the U.K. but can affect all tissues of the body. Most commonly the babies have meningitis or renal involvement, although pulmonary disease, joint infection, and infective endocarditis are well recognized. They often present in the first 10 days of life, but later-onset disease may be associated with the long-term use of broad-spectrum antibiotics. The clinical appearance is similar to bacterial sepsis, with *Candida* being isolated from the blood, cerebrospinal fluid (CSF), or joint fluid.

Candida can be found in the sputum, urine, and feces in the absence of invasive disease. Transient candiduria may occur in patients on

antibiotics, especially if they are catheterized. Persistent funguria after removal of catheters, and particularly if the urine is obtained by suprapubic aspiration, may indicate systemic or urinary tract candidiasis. With renal involvement, ureteropelvic obstruction can occur because of fungal masses. These can be detected using ultrasound. Mortality is high in disseminated disease, but the use of prophylactic antifungal treatment to prevent disease in at risk groups is unproven.

Viral infection can be a problem in the preterm infant. Cytomegalovirus reactivation during lactation has been shown, and breast-feeding as a source of symptomatic postnatal cytomegalovirus infection in preterm infants may well be underestimated (Hamprecht et al. 2001).

Outcome for the Extremely Preterm Infant

Differences in study design make it difficult to compare many of the follow-up studies of preterm infants. Most are hospital- rather than population-based and reflect practice within a single institution. Many studies include only live births, but attitudes to the registration of very immature infants, who show only transient signs of life after birth, are not always consistent (Fenton et al. 1990). Gestation is the major determinant of outcome. In many cases the actual gestational age is uncertain, and at the limits of viability even minor changes may have a major impact on outcome statistics. Neonatal mortality can be influenced by the vigor with which these babies are resuscitated after delivery, while intensive care may merely delay death.

The EPICure study collected data, during 1995, on all births in the British Isles between 20 and 25 weeks' gestation (Costeloe et al. 2000). Of a cohort of 4004 births, 811 (20%) were admitted for intensive care and of these, 314 (39%) survived to their expected date of delivery. Survival at 22, 23, 24, and 25 weeks was 9%, 21%, 36%, and 54%, respectively, of those admitted. Improved outcome was associated with the use of antenatal steroids but not with surfactant replacement. Parenchymal cysts or hydrocephalus were found in 17% of the survivors.

Follow-up data from this study, at a median of 30 months corrected age, showed that 50% of survivors had some disability, with 23% meeting the criteria for severe handicap (Wood et al. 2000). By 6 years of age the rates of severe, moderate, and mild disability were 22%, 24%, and 34%, respectively. Disabling cerebral palsy was present in 12%. Of children with severe disability at 30 months of age, 86% still had moderate to severe disability at 6 years of age. Other disabilities at age 30 months were poorly predictive of developmental problems at 6 years of age (Marlow et al. 2005). Boys are disadvantaged compared to girls, in mortality, morbidity, and developmental outcome.

In these very immature infants, cognitive and academic problems persist into adulthood, as do attention, peer, and possibly emotional problems, and employment prospects are somewhat poorer. In the less immature babies, postdischarge interventions are more likely to be of benefit and alter long-term outcome. Avoiding extremely preterm birth, and optimal neonatal care that reduces secondary damage, may be the key intervention for those threatening to deliver or who are born extremely premature. In contrast, postdischarge educational/family interventions may benefit mostly those born only slightly preterm or of low birth weight.

The limit of viability is largely determined by lung maturity. Improvements in outcome have been seen with antenatal steroids and replacement surfactant, although there are few data to show that these treatment modalities have made any difference to gestations below 25 weeks. The respiratory system is probably the last to achieve functional maturity, and until new methods of supporting the very immature lung are found it is unlikely that we will achieve much further improvement in outcome at the extremes of viability.

Quantitative magnetic resonance techniques have been used to investigate subtle differences in cerebral growth and development among children and adolescents born preterm or with very low birth weight. These imaging tools include diffusion tensor imaging and computer-assisted magnetic resonance techniques for measuring cortical folding and regional brain volume. When compared with the baby born at term, differences have

been shown in the development of white matter, as well as cortical and subcortical gray matter, in the preterm infant (Counsell and Boardman 2005). Some of the alterations have been shown to have specific functional correlates. Although brain volume in preterm infants at term was similar to that of infants born at term, the surface area of the cortex and cortical folding was reduced in preterm infants. These techniques are providing evidence that, even in the preterm infant who appears to do well, there are difference in brain development when compared with the baby born at term. Several studies show that morphometric changes persist into adolescence, and that these correlate with functional impairment.

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12
Pathology of Twinning

Robert W. Bendon

Twin pregnancies have increased rates of premature labor, pregnancy-induced hypertension, breech lie, cerebral palsy, and perinatal death, among other complications (Kovacs et al. 1989). Some of these complications are due to the poor adaptation of the human uterus to the increased demands and mass of twin pregnancy. Some complications are related to the obstetrical problems of delivering twins, although modern obstetric management greatly reduces these risks. This chapter focuses not on these general problems but on those associated more specifically with the formation of twins. The discussion focuses on twins as the simplest unit of multiple gestation. The same considerations usually apply to higher multiples of gestation—triplets, quadruplets, etc. These multiple gestation pregnancies have become more common with the increased use of assisted reproduction techniques (Callahan et al. 1994).

Zygosity and Chorionicity

Monozygosity is the state of twins formed from a single fertilized ovum. Dizygosity is the formation of two separate twins from two separate fertilized eggs. The rate of dizygotic twin gestation can vary with ethnic group and geographic location from 1% to 50% of pregnancies (MacGillivray 1986). Normally ovulation of one oocyte suppresses the others, and a single ovulation occurs. Fertility therapy with exogenous gonadotrophins results in increased oocyte release and multiple gestation. The increased incidence of dizygotic twins in some populations and families similarly has been associated with elevated follicle stimulating hormone secretion (MacGillivray 1986).

Dizygotic twins have a complete chorionic membrane surrounding each embryo; that is, they are dichorionic. The parenchyma of the placentas may fuse or remain separate. Each twin circulates blood to its own placenta. There is one published exception of dizygotic twins resulting from in vitro fertilization having fused to produce a monochorionic twin placenta (Souter 2003).

Monozygotic twinning has a uniform rate across populations (3% to 5%). The varied placentation of monozygous twins implies that the zygote may duplicate at different stages of development. If it divides before cells are segregated to form the placenta, the placenta will be dichorionic. Most commonly, the division of the embryo occurs after commitment of trophoblastic cells but prior to formation of the amnion. The placentation will be diamniotic and monochorionic. Rarely twinning occurs after amnion formation and the placenta is monoamniotic and monochorionic.

Pathological examination of the placenta can determine the chorionicity. In a dizygotic twin, the placenta will be either separate or fused dichorionic. Separate placental disks are dichorionic and are likely to be, although not always, dizygotic (Fujikura and Froehlich 1971). The placenta of monozygotic twins may be dichorionic or monochorionic. To determine chorionicity in a fused placenta, the septum (the membrane between twin sacs) must be identified. A dichorionic septum usually has a small ridge where it turns up from the placental surface (the ultrasound delta

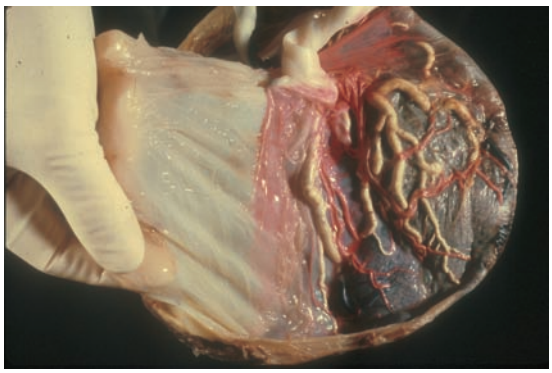

Figure 12.1. The septum is opaque in this fused dichorionic twin placenta.

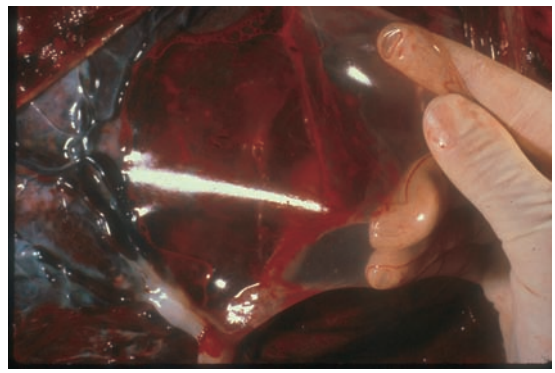

Figure 12.3. The septum in this diamniotic monochorionic twin placenta is completely transparent.

sign) and will have some opacity (Fig. 12.1). A microscopic section of a dichorionic septum shows either fused or abutting chorion epithelial layers (Fig. 12.2) between two amnions. The chorion layers may fuse or be separated by amorphous eosinophilic material. A monochorionic placenta has no septum or a transparent septum (Fig. 12.3). The septum in a monochorionic placenta has two layers of amnion and no chorion (Fig. 12.4). Some authors have recommended taking a T-section, that is a sample that includes the septum as well as the underlying placenta (Allen and Turner 1971). If the septum is confidently identified, it is sufficient alone to determine chorionicity. Higher multiples of gestation require examining the septal relationship between each pair of siblings.

Birth Weight Discordance

A 15% (or 20%) difference in birth weight between twins is defined as discordance. Dizygotic twins may have genetic or chromosomal causes of birth weight difference. Monozygotic twins usually, but not always, are genetically identical. They may be subject to birth weight differences inherent in the process of splitting the zygote (Machin 1996). Monochorionic twins may also have birth weight differences due to complications of the shared placental circulation. Independent of zygosity, any of the etiologies of intrauterine growth

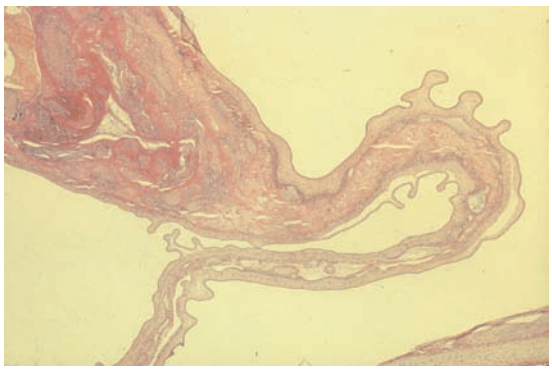

Figure 12.2. This lower-power micrograph of the septum of a dichorionic twin placenta demonstrates the thickening often seen at the insertion into the placenta, as well as the distinct chorionic tissue between the amnion layers.

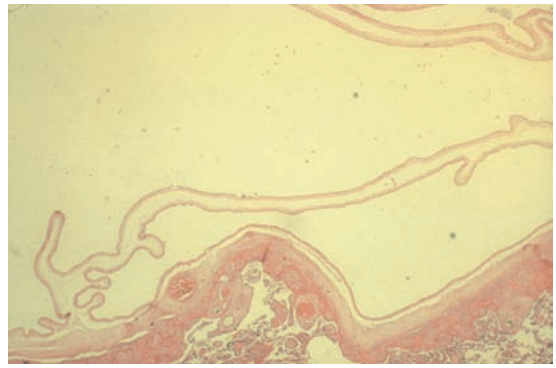

Figure 12.4. This lower-power micrograph of a diamniotic septum arising from the surface of the placenta demonstrates two simple amnion layers with no chorionic tissue between them. The appearance of two amnion layers can be created by a fold in a single amnion layer. The pathologist needs to be certain that the septum has been sampled for the microscopic section.

restriction present in singletons may be present asymmetrically in twins (Wenstrom et al. 1992). Less optimal implantation, for example, over a leiomyoma or over the lower uterine segment, may also affect intrauterine growth. The investigation of discordant twin growth needs to consider fetal karyotypic and anatomical anomalies, as well as placental lesions.

Vanished Twin

Dizygotic twins are genetically distinct individuals with dichorionic placentas without vascular connections. Chromosomal errors are common causes of first gestation fetal loss in singletons, and twins may have an even higher rate of loss. One twin of a dizygotic pair may die early in gestation without interfering with the continuation of a normal pregnancy for the other twin. Obstetricians have referred to this as the vanished twin syndrome. The later in gestation that one twin dies, the more likely that the "vanished twin" will be found by placental examination. If the embryo has developed a small placenta, a disk of yellow thickening may be found in the membranes (Sulak and Dodson 1986) (Fig. 12.5). The gross appearance is more compact and the color is less tan than in areas of decidual necrosis or old hematoma, and the mass is larger and less white than the typical yolk sac. Microscopic examination will show ghosted chorionic villi from early gestation. A radiograph or a microscopic section may also identify fetal tissue (Fig. 12.6).

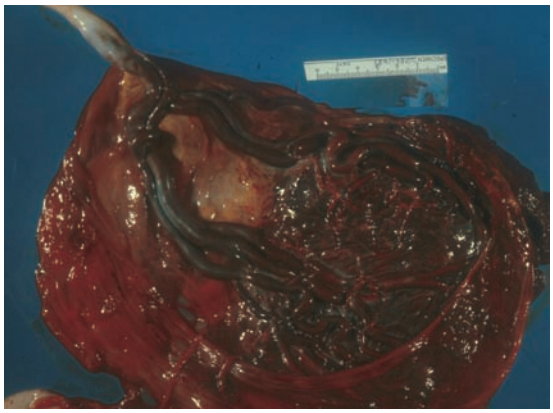

Figure 12.5. The pale tissue beneath the amnion and between the velamentous vessels of the cord insertion is the remnant of an early twin death.

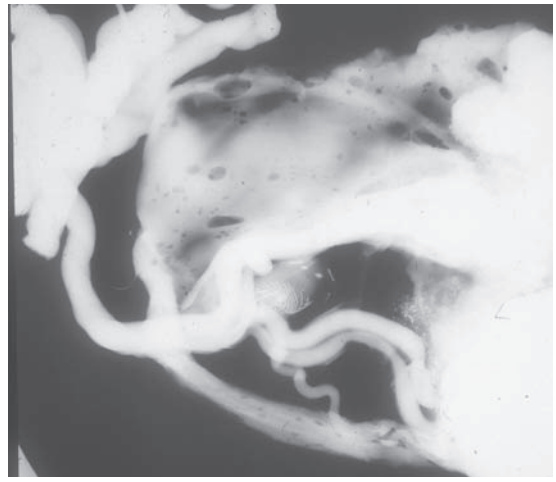

Figure 12.6. This radiograph of the same placenta photographed in Figure 12.5 demonstrates the small fetal skeleton of the dead twin.

A larger compressed fetus, fetus papyraceous, may provide a placental septum to be sampled to confirm the chorionicity. The gestational size (crown–rump, foot length) as well as major external malformations can be noted. The placental examination shows secondary effects of prolonged retention, for example, villous sclerosis and extensive perivillous fibrinoid.

Malformations in Monozygotic Twins

Malformations are increased in monozygous twins compared to singletons or dizygotic twins (Fogel et al. 1965). The mechanisms of malformation have been categorized as processes inherent in twinning; processes secondary to the vascular connections in the placenta, particularly with death of one twin; and deformations associated with crowding in utero (Schinzel et al. 1979). The first set of malformations includes conjoined

twins and cardiac anomalies from disturbed laterality during development (Burn and Corney 1984). A fourth group of discordant malformations arises when the twins have unequal distribution of a chromosomal defect. A striking example is that of monozygotic twins discordant for gender due to loss of Y chromosome material, usually producing a male and a Turner phenotype female (Yokota et al. 1994). The malformations from all these processes are often discordant between twins.

Conjoined Twins

Conjoined twins may be complete or partial with extra portions of the body axis incorporated into one individual. Experimentally, conjoined twins can be produced by various insults to the cleavage stage zygote prior to gastrulation (Stockard 1921). The traditional classification of the anatomical connections and the approximate percentage of each is shown in Table 12.1 (Filler 1986). The autopsy of conjoined twins may demonstrate the anatomical abnormalities that would have presented surgical difficulties such as fusion of the heart chambers or of the brain ventricular system. Of scientific interest are cardiac lateralization defects similar to asplenia syndromes that occur in separate monochorionic twins, but more commonly in thoracopagus twins (Noonan 1978; Harper et al. 1980; Cunniff et al. 1988) (Fig. 12.7). The pathologist may be asked to distinguish a parasitic partial twin from a sacral teratoma or cranial malformation. The partial twin has a body axis and some organ formation (Drut et al. 1992).

Table 12.1. Types of conjoined twins

Type	Anatomy	Approximate percentage
Craniopagus	Joined at the head	2%
Thoracopagus	Joined at the chest	75%
Xipho- or omphalopagus	Joined at the abdomen (included with thoracopagus)	
Ischiopagus	Joined at the pelvis	5%
Pygopagus	Joined at the sacrum, facing away from each other	20%

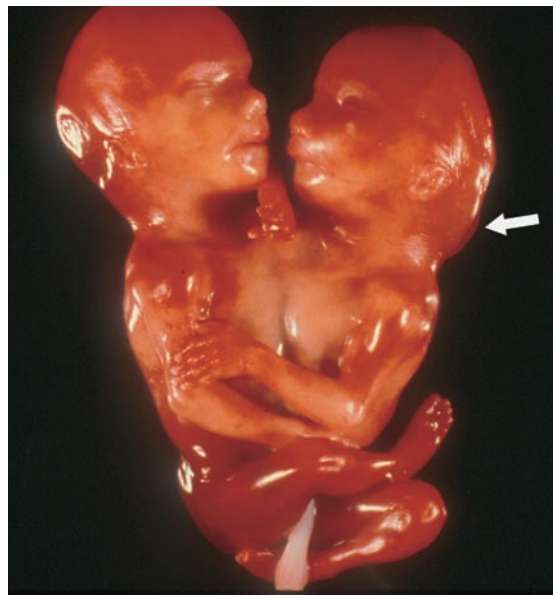

Figure 12.7. These conjoined thoracopagus twins at 17 weeks' gestation had a single pelvis and lower limbs. The heart of the twin with the arrow had multiple small spleens, bilateral left atrial appendages, and common right ventricle, bradycardia, and fetal hydrops with posterior nuchal edema (arrow).

Monochorionic Placentas and Their Complications

A monochorionic placenta, in effect a conjoined placenta, creates vascular connections between twins. The placental vessels form separately from the fetal vessels to which they subsequently connect via the body stalk vessels (the future umbilical cord). Branching arteries and veins on the placental surface enter (or exit) the parenchyma as end vessels of stem villi. One inter-twin vascular connection, referred to as arteriovenous anastomosis, is created when an artery from one twin and a vein from the other twin vascularize the same stem villus (Fig. 12.8). This anatomy shunts blood from one twin to the other at a rate determined by normal villous vascular resistance. Usually there are multiple such shunts in both directions for which the net shunt of blood is a simple vector addition. The other two possible inter-twin connections are artery-to-artery and vein-to-vein anastomoses on the chorionic surface (Fig. 12.9). These have the potential to create shunts through stem villi that vary with the hemo-

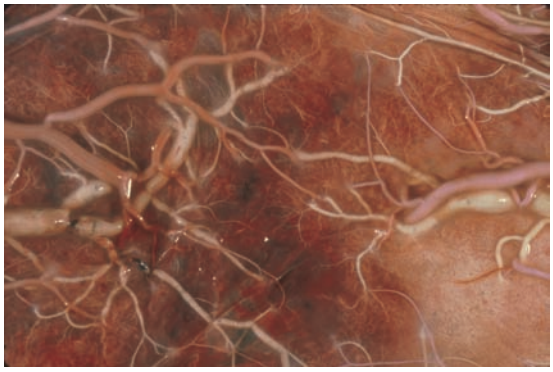

Figure 12.8. This photograph of a vascular injection of a monochorionic twin placenta at the vascular equator demonstrates many arteriovenous anastomoses. In the center dark arteries from the twin on the left (arteries cross over veins) are entering the parenchyma with light-colored veins from the twin on the right.

dynamic parameters (resistance, pressure, and flow) relative to each twin. If arterial blood from one twin is pushed past the vascular equator, it begins to flow into the venous system of the other twin. Such anastomoses can help balance the fixed shunt created by arteriovenous anastomosis. The end on artery-to-artery flow can be visualized as having a fixed neutral point where both arterial flows stop. This point can move along the arterial anastomosis depending on the relative blood pressures of the twins. In a simplified model, a fixed transfusion raises blood pressure of the recipient and lowers that of the donor. This moves the neutral point further into the donor's villi, resulting in a balancing transfusion in the opposite direction. Some monochorionic placentas have no vascular connections, but all others are at risk for complications from these vascular connections.

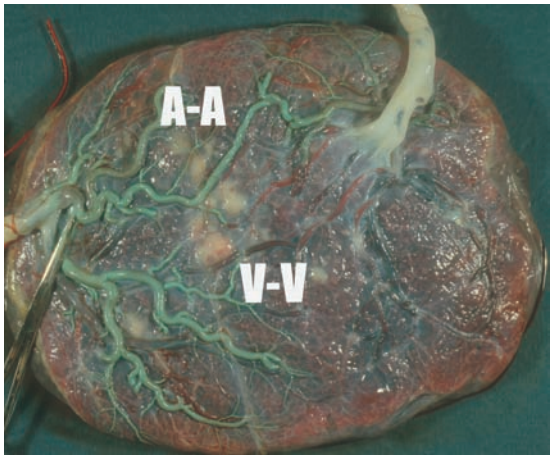

Figure 12.9. This placenta had a single arterial injection, which has filled the arterial tree of both twins. The artery-to-artery anastomosis can be seen below A-A. There is also a darker vein-to-vein anastomosis that can be seen above V-V.

One such complication is the twin-to-twin transfusion syndrome (TTS). If the chorionic circulation cannot balance between twins, there is a continuous transfusion from one twin to the other. If this transfusion is large, it leads to death from exsanguination of the donor twin. In cases with smaller net transfusions, stability can be established if the initial transfusion leads to adaptive responses of the hydrodynamic and osmotic pressures to increase resistance in the shunt circuits and minimize or eliminate blood shunting (Talbert et al. 1996). The hydrodynamic changes are manifested in discordant fetal and placental growth, and in cardiac hypertrophy in the recipient twin. The osmotic adaptations result in polyhydramnios in the recipient twin and oligohydramnios in the donor twin. The latter produces the stuck-twin appearance on prenatal ultrasound, which is a key diagnostic feature of TTS. The polyhydramnios may lead to preterm labor, which can be delayed by repetitive amniocentesis (Mahony et al. 1990; Urig et al. 1990). Amniocentesis does not eliminate the underlying pathophysiology of TTS, which can eventually result in heart failure in the recipient twin and chronic hypotensive ischemic injury in the donor. Either twin may develop heart failure or death. Selective in utero coagulation of chorionic vessels offers the promise of more definitive cure by separating the placental circulations (De Lia et al. 1999; Quintero et al. 2001; Senat et al. 2004).

The stability between the twins in TTS is jeopardized by any event that changes the hemodynamic balance between twins. This imbalance permits the unbalanced transfusion of blood from one twin to the other, often evidenced by plethora in the recipient twin and pallor in the donor. This acute transfusion can occur in any set of twins with a shared placental circulation. In TTS, the onset of cardiac failure in one of the twins, or the inability of the small donor twin, often with a

small placenta, to tolerate the intermittent uterine ischemia of labor may tip the balance to acute twin transfusion. Other factors, such as thrombi in the turbulent artery-to-artery anastomosis or any of the events that can cause prenatal or intrapartum fetal distress, can also initiate acute transfusion (Nikkels et al. 2002; Tan et al. 2004). If not interrupted by delivery, an acute transfusion can result in a return to the previous steady state, a new steady state, or a continuous imbalance until one or both twins die.

In most cases of the death of both monochorionic twins in utero, one twin is deeply plethoric and the other pale (Bendon 1995). Logically, if one twin dies first, that twin will be a sink for blood from the surviving twin via the placental anastomoses (Fig. 12.10). The direction of the transfusion may be the same or opposite that of TTS if present in the same twins. The transfusion may be so large as to reverse the body weight, with the chronic donor twin becoming heavier than the chronic recipient twin due to the acute transfusion in the opposite direction (Bendon and Siddiqi 1989). In the less common case in which one twin survives, that twin is at risk of ischemic injury resulting from the hypovolemic shock that results from transfusing the dead sibling (Fusi et al. 1991). The surviving fetus consistent with the acute donor status is often anemic within 24 hours of fetal death (Senat et al. 2003). The ischemic lesions include aplasia cutis, bowel atresias and central nervous system lesions (Hoyme et al. 1981). In liveborn twins, vein-to-vein anastomoses have been associated with an increased risk of cerebral lesions, perhaps because venous pressures are less stable than arterial pressure and more likely to result in acute transfusion (Bejar et al. 1990).

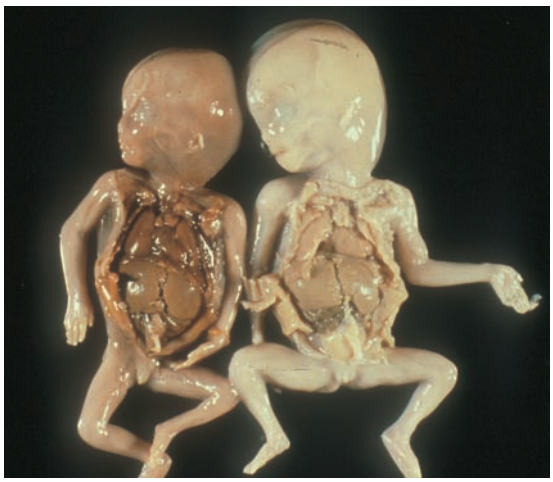

Figure 12.10. These monochorionic twins died in utero. The smaller twin even after prolonged postmortem intrauterine retention shows the plethora from the acute twin transfusion from the paler, larger twin.

In TTS, both recipient and donor surviving twins have a high incidence (25%) of brain abnormalities identified by ultrasound (Grafe 1993; Mari et al. 2001). Chronic TTS does not require an actual steady transfusion of blood, only a stable state; therefore, hematocrit differences between twins are small (Saunders et al. 1991). In a study of repetitive amniocentesis to treat TTS, a hematocrit difference was associated with a greater risk of neurological abnormality on imaging studies (Mari et al. 2001). This hematocrit difference could be a marker of an episode of acute twin transfusion.

The pathologist's examination of the placenta begins with confirming monochorionicity and doing a routine pathological examination similar to that in any singleton placenta. The velamentous insertion of the umbilical cord and even the pattern of vascular distribution on the placental surface may correlate with the risk of anastomoses (De Paepe et al. 2005). The direct artery-to-artery and vein-to-vein anastomoses can be counted, and their approximate diameter recorded. The surface arteries cross over the veins. There is some evidence that small artery-to-artery anastomoses may be protective of chronic TTS by balancing inequalities in fixed shunts (Bajoria et al. 1995; Machin et al. 1996; Denbow et al. 2000; Umur et al. 2002). The typical artery-to-vein anastomoses through the parenchyma may be visible at the "vascular equator," which is where the two circulations meet. The pairing of an artery from one twin with a vein from the other may be evident. This simple external description may not reflect the full extent of anastomoses as the division of vessels may continue beneath the visible surface (Wee et al. 2005). Aids to observation of the vascular anatomy have traditionally included the intravascular injection of air, milk, wine, latex, plastic polymers, or barium. A continuous flow with low-viscosity colored perfusion media can

reveal details of arterial venous connections. More viscous or polymerizable material will allow dissection of the deeper vessels. In most cases a description of the surface vessels as observed by the pathologist with minimal manipulation, such as pushing blood with a finger on the surface of the placenta to confirm anastomoses, is sufficient. A detailed quantitative description, given our current understanding, cannot solely be used to diagnose TTS and therefore is not of diagnostic value. In cases with laser ablation of vessels, the coagulated areas often show extensive villous necrosis and obscured vascular anatomy (De Paepe et al. 2004). Recording the number and location of laser-produced lesions and the respective placental mass of each twin aids in the evaluation of the laser procedure (Quintero et al. 2005).

In placentas without vein-to-vein anastomoses, the relative volume of venous return to each twin may correlate with their size discordance (Machin et al. 1996). In cases of long-standing transfusion, both the gross examination and the microscopic evaluation may demonstrate marked differences in villous vascularity (Pietrantoni et al. 1998). Similarly, with long-standing oligohydramnios of one twin, amnion nodosum may develop in that twin's sac (Fig. 12.11). In acute transfusion, the plethora and pallor of the twins is reflected in the villi. If there is fetal hydrops, the affected twin's placenta is likely to be hydropic.

The autopsy of twins with TTS can demonstrate the long-standing effects of hypoperfusion in the donor twin. The kidneys may show renal tubular dysplasia, and there may be general atrophy of internal organs (Pietrantoni et al. 1998). The donor frequently shows cardiomegaly, endocardial sclerosis, and evidence of cardiac failure (Fig. 12.12) (Barrea et al. 2005).

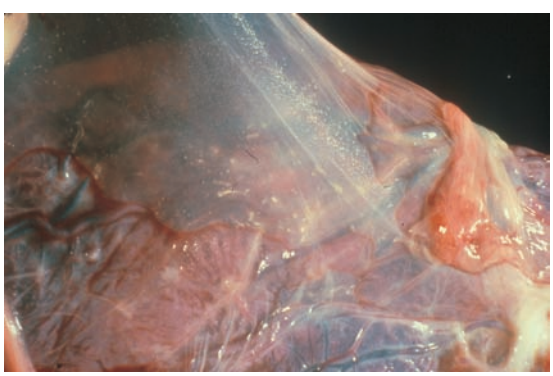

Figure 12.11. The septum of this monochorionic placenta with prolonged chronic twin-to-twin transfusion demonstrates fine amnion nodosum due to the prolonged oligohydramnios.

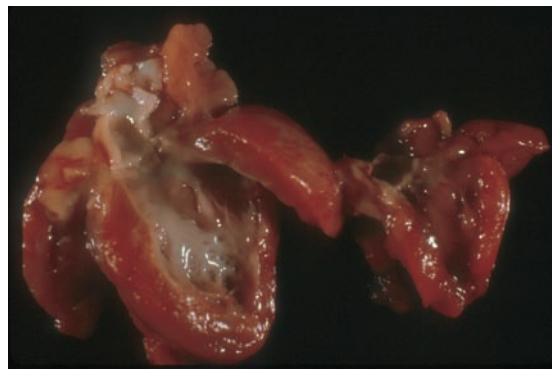

Figure 12.12. The dissected hearts of 20-week-gestation twins from chronic twin-to-twin transfusion show a marked difference in size. The ratio of the larger twin to smaller twin heart weight was 4.4. The ratio of the larger twin to smaller twin body weight ratio was 1.5. The larger recipient twin heart demonstrates a thickened endocardium, a reflection of increased outflow pressure.

Acardiac Twin or Twin Reversed Arterial Perfusion

This entity occurs when the donor twin provides the entire circulation of the recipient twin through a monochorionic placenta (Van Allen et al. 1983). This concept can be imagined as follows: During early development the blood pressure of a recipient twin is much lower than that of the donor. Arterial blood from the donor can then enter the umbilical arterial circulation of the recipient. If the donor's blood pressure exceeds the recipient's systolic ventricular pressure, the recipient's heart could not pump blood and would atrophy. The arterial blood under the donor's pressure would enter the venous system of the recipient and eventually drain in a retrograde fashion into the umbilical vein and placenta. The survival of the recipient would then depend on this retrograde, low-oxygen flow.

The etiology of the pressure difference could be from cardiac malformation in the recipient or unequal distribution of a chromosome anomaly (Moore et al. 1987), or perhaps even late develop-

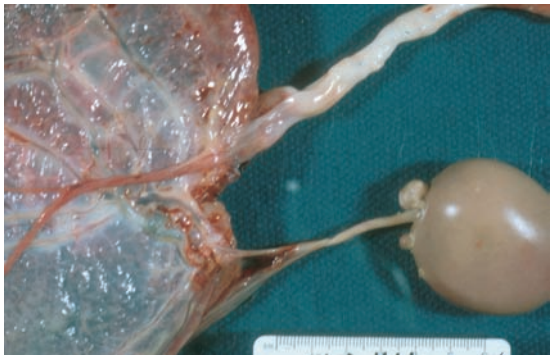

Figure 12.13. The rounded mass attached at the placental periphery proved to be an amorphous acardiac twin.

ment of a connection to the placenta. Once established, the retrograde circulation has several consequences. There is extensive organ reduction due to ischemia (Gimenez-Scherer and Davies 2003). At the extreme, the acardiac twin may have little more than a simple body axis, an acardius amorphous. There is often marked tissue edema. If the acardiac twin is large, the increased cardiac work may cause cardiac failure in the donor (Moore et al. 1990).

The pathologist may be called to distinguish the amorphic acardiac fetus from a neoplasm. This often occurs when a mass is attached to the distal vessels of the placenta without a definite umbilical cord (Fig. 12.13). The parasitic relationship to the placental circulation, as well as preservation of a body axis, confirms the diagnosis (Fig. 12.14). In the case of a more completely formed twin, confirmation of the reversed circulation and acardia (or a very hypoplastic heart remnant) is diagnostic (Fig. 12.15). Obtaining tissue for karyotype from the acardiac twin may demonstrate a chromosomal etiology.

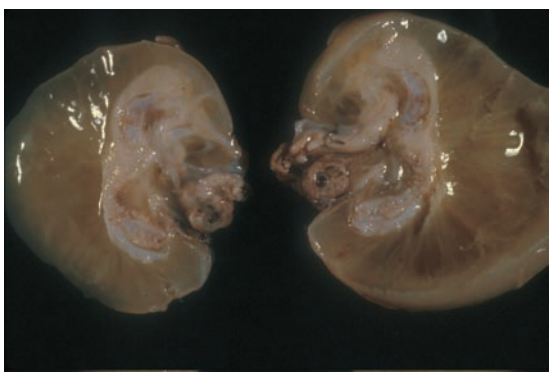

Figure 12.14. A mass similar to that in **Figure 12.13** has been bivalved, revealing an embryonic axis and partial organ formation.

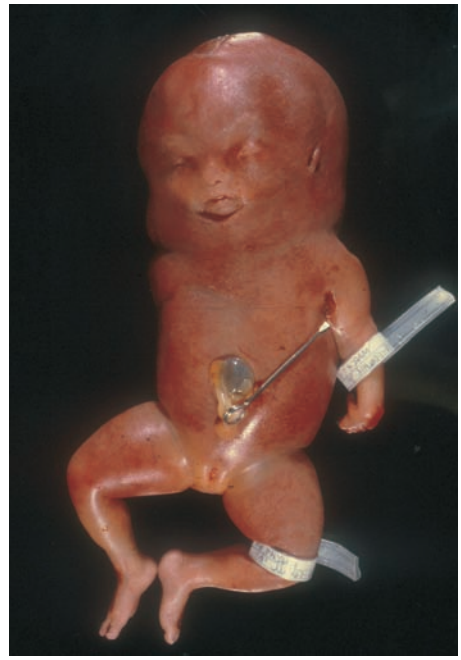

Figure 12.15. A more completely developed acardiac twin shows edema and the loss of one arm. This infant had limb movement immediately after cesarean birth.

Monoamniotic Twins

Monoamniotic twins are subject to all of the complications of a diamniotic monochorionic twin pregnancy. A unique complication of sharing a single sac is the entanglement of the umbilical cords (Fig. 12.16). Such a tangle might knot or kink a cord and, even if not completely occlusive, could initiate an acute twin transfusion and death of one or both twins (Golan et al. 1982). Monoamniotic twins generally have umbilical cords that are close together on the placental surface, often with large artery-to-artery and vein-to-vein anastomoses. This anatomy results in equal sharing of the placental circulation and less likely chronic twin-to-twin transfusion, but entails more risk for acute twin transfusion (Umur et al. 2003).

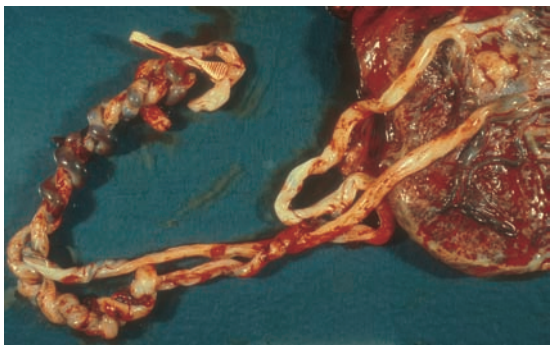

Figure 12.16. An entangled umbilical cord from liveborn monoamniotic twins.

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13
Intrapartum Problems

Jean W. Keeling

The past 50 years has seen major changes in the organization and level of provision of obstetric services in Western Europe, North America, and Australia and New Zealand. Because of this, and other factors, such as improved population health, education, and housing, and advances in other areas of medicine such as blood transfusion and the management of diabetes, the perinatal mortality rate (PMR) fell rapidly in the second half of the 20th century. In the United Kingdom in 1958, the British Perinatal Mortality Survey reported a PMR of 33.2/1000 total births (Butler and Bonham 1963); it is currently <8/1000 total births [Confidential Enquiry into Maternal and Child Health (CEMACH) 2005]. The fall in intrapartum-related deaths has been even more dramatic, 10.2/1000 in 1958 compared to 0.53/1000 in 2002 (CEMACH 2005).

The effects of this decrease in intrapartum-related mortality are many. With so few deaths, the public perception is that there is no longer any risk to a mature baby from intrapartum events, to the extent that any intrapartum death must be someone's fault. An intrapartum death engenders difficulties for the obstetricians and midwives involved, even when procedures were entirely appropriate to the circumstances. The decrease in intrapartum-related mortality also means that pathologists, even those working with specialized or regional obstetric units, have little experience of these deaths, particularly where there is trauma.

In the developing world, PMRs are higher and the contribution to the total from intrapartum events approaches that seen in the U.K. in 1958. Indeed, in Jamaica in the late 1980s, intrapartum events contributed to 44% of perinatal deaths at a time when the PMR was 38/1000 (Ashley et al. 1994). More recent information from South Africa reported PMRs of around 35/1000, with underlying intrapartum events identified in 6.92/1000 in rural areas (Pattinson 2003).

If the contribution of intrapartum events, whether hypoxia/ischemia or trauma, to PMR is to be accurately identified, so that appropriate lessons are learned, then a high necropsy rate is essential. In the U.K. in recent years, postmortem rates have fallen short of the 75% suggested as a minimum by a Joint Working Party of Royal Colleges (1988). When intrapartum management is even remotely considered to be a responsible or contributory factor in perinatal death, it is important that every effort be made to ensure that authorization for necropsy is obtained and that it is performed by a pathologist with appropriate experience and training.

When death is thought to be intrapartum related, it is important that the postmortem examination is of the highest possible standard. Just because the possibility of intrapartum problems has been considered relevant and even when these seem likely, all investigations, including examination of the placenta and of the brain after fixation, together with microbiological investigations and histological sampling of all organs, must be carried out (see Chapter 2). Examination of the cranial cavity should be done with care, so that important structures such as dural sinuses and folds are not damaged by the examination itself.

This chapter is based in part on Chapter 10 "Intrapartum Asphyxia and Birth Trauma" written by Iona Jeffrey in Fetal and Neonatal Pathology 3rd Edition. Copyright Springer-Verlag, London 2001.

The commonest conditions that may be confused clinically with intrapartum hypoxia/ischemia and trauma are ductus-dependent cardiovascular malformations, respiratory tract malformations, such as diaphragmatic hernia and nonsyndromic laryngeal atresia, intrauterine septicemia/pneumonia, particularly group B streptococcal infection and antepartum hypoxic/ischemic brain damage. Acute fetomaternal hemorrhage is a less common mimic of intrapartum damage and is best excluded by Kleihauer's method to detect fetal red blood cells in the maternal circulation. It is a standard investigation following stillbirth in obstetric units in the U.K. If death follows sudden deterioration in the early neonatal period, it is important to exclude ductus-dependent cardiac lesions and genetic metabolic disease.

When compiling the necropsy report following intrapartum-related death, it is most important to check carefully for typographical and other errors (including decimal points), that descriptions are precise and that negative findings are clearly set out. The language used should be non-emotive and abnormal findings appropriately attributed. The cause and contribution of any traumatic lesion should be carefully evaluated. The clinician should keep in mind that a copy of the report is likely to be handed to the parents and subsequently may be scrutinized by lawyers.

Intrapartum Asphyxia

Definition

Intrapartum asphyxia is a condition of impaired gas exchange occurring during labor, leading to progressive hypoxemia, hypercapnia, and significant metabolic acidosis in the fetus (Low 1997). During normal labor, there is usually a slight reduction in blood pH and oxygen concentration in healthy, full-term infants. Diagnosis of intrapartum asphyxia requires levels of these parameters that are out of the normal range. Fetal scalp blood, obtained during labor, or cord blood taken immediately after delivery in liveborns, are suitable samples. Commonly used criteria for intrapartum asphyxia are a base deficit of between 12 and 16 mmol/L and a pH <7.2 (Low 1997). However, it is evident that biochemical disturbances in fetal blood gases do not give the whole picture and correlate poorly with neonatal outcome (Ruth and Raivio 1988; Dennis et al. 1989; Goodwin et al. 1992). A single biochemical measurement does not allow assessment of the severity of the asphyxial insult because it does not reflect important variables such as the duration of acidosis, and whether the asphyxial episode recorded just before delivery was unique. Babies who suffer an asphyxial insult early in labor may recover to the extent that the pH and base deficit are within limits at delivery, despite prior organ damage (Chiswick 1993; Goodlin 1997). Conversely, severely abnormal cord blood gases and pH may reflect a brief episode of hypoxemia at the end of the second stage of labor, easily correctable after birth and without sequelae (Casalaz et al. 1998). This has led to proposals to widen the definition of birth asphyxia to include infants who not only have metabolic acidosis but also show clinical evidence of brain or other organ damage (Murphy et al. 1990; Thorp 1990), or even abandon the term altogether (Johnson 1993).

Incidence

Routine blood gas and acid–base measurements in cord blood at delivery reveal that around 2% of babies have a base deficit >12 mmol/L in umbilical arterial blood, and are deemed to have suffered a significant asphyxial episode during labor and delivery (Low 1997). Although such infants may require some resuscitation at birth, most respond promptly to bagging, and there are no long-term sequelae. Severe intrapartum asphyxia leading to neurodevelopmental handicap or death is much less common, affecting less than 0.5% of infants, and is poorly predicted by the level of hypoxia and acidemia at birth (Casalaz et al. 1998).

Although intrapartum asphyxia is well recognized in full-term infants, it affects preterm babies more commonly than is diagnosed either clinically or postmortem (Low et al. 1990).

Pathophysiology

During normal labor, contractions compress the uterine blood vessels, with intermittent interruption to blood flow to the placenta. Following membrane rupture, uterine volume decreases and

there is a reduction in the volume of the intervillous space that further diminishes placental perfusion. At the height of a contraction there is no oxygen delivery to the fetus. In normal labor, this is rapidly reversed between contractions when the uterus relaxes and delivery of oxygenated blood is restored.

Studies of normal labor, with a healthy infant and placenta, show only a slight fall in the mean pH to between 7.23 and 7.29 (Spencer 1993). Abnormalities in the mother, placenta, or infant that prolong labor, result in excessive or prolonged head compression, or prevent successful adaptation to the effects of uterine contractions result in fetal hypoxemia, which, if sustained, leads to acidemia and asphyxia.

Fetal responses to intrauterine asphyxia have been studied in a variety of animal models using different methods to induce hypoxemia and acidemia (Painter 1995; Roohey et al. 1997). Studies in sheep have shown that a reduction in partial pressure of oxygen is sensed by fetal arterial chemoreceptors that stimulate the autonomic nervous system (Giussani et al. 1993), resulting in peripheral vasoconstriction with redistribution of blood flow to the brain, heart, and adrenals (Reid et al. 1991). A moderate degree of hypoxemia is tolerated for some time, but if it persists, a switch to anaerobic metabolism results in production of increasing amounts of lactate (Giussani et al. 1993). Failure to adequately remove lactate and carbon dioxide promotes fetal acidosis. Similar changes operate in the human fetus (Berger and Garnier 2000).

Effects

Mature infants have a capacity to resist the adverse effects of hypoxia and acidosis for a short time. However, prolonged periods of low oxygen concentration and acidosis have important adverse effects on the fetal circulation, fetal breathing movements, brain, kidneys, and other organs. If delivery is not expedited, there is severe and irreversible damage to susceptible organs and, ultimately, cardiac arrest and death. If the infant is liveborn, the severity of the intrapartum asphyxia can be assessed by the development of clinical complications including abnormalities of cardiac rate and rhythm, blood pressure, respiratory complications, renal failure, and fits or other abnormal neurological signs (Chiswick 1993).

Effects on the Fetal Circulation

The cardiac response to hypoxia is bradycardia. Intrapartum asphyxia is usually first suspected during labor by abnormalities of the fetal heart rate (Steer and Danielian 1994). Under normal circumstances the fetal heart rate is between 120 and 160 beats per minute (bpm). However, the rate changes from one minute to the next, baseline variability generally being between 5 and 15 bpm. Superimposed on this there are normally small accelerations of greater than 15 bpm for more than 15 seconds every few minutes (Fig. 13.1A). The presence of fetal heart accelerations is a reassuring finding, indicating absence of acidemia, and a normal baseline rate with normal variability during contractions implies adequate levels of fetal oxygenation are being maintained. Reduced baseline variability, absent accelerations, increased or decreased average heart rate may all indicate fetal distress. Transient decelerations of fetal heart rate during the early phase of uterine contractions are common in the second stage of labor. They are probably mediated by a vagal reflex in response to compression of the head and not associated with significant fetal compromise. In contrast, long or repeated decelerations in the late phase of uterine contractions with slow recovery may reflect fetal asphyxia (Milsom et al. 2002), especially when combined with a baseline tachycardia or bradycardia (Fig. 13.1B). Fetal heart rate abnormalities are not diagnostic of asphyxia. Diagnosis requires fetal blood sampling and demonstration of a significant reduction in fetal blood pH (Steer and Danielian 1994). Intrapartum hypoxia damages capillary endothelium and produces a generalized loss of muscle tone within the microcirculation, leading to intense generalized congestion of tissues and organs.

Effects on Fetal Breathing

Studies on fetal lambs rendered hypoxic during labor show that as the degree of hypoxia and acidosis increases, breathing movements are reduced and then cease. A period of primary apnea (Patrick et al. 1976) is followed by deep, sighing respirations before cessation of breathing movements

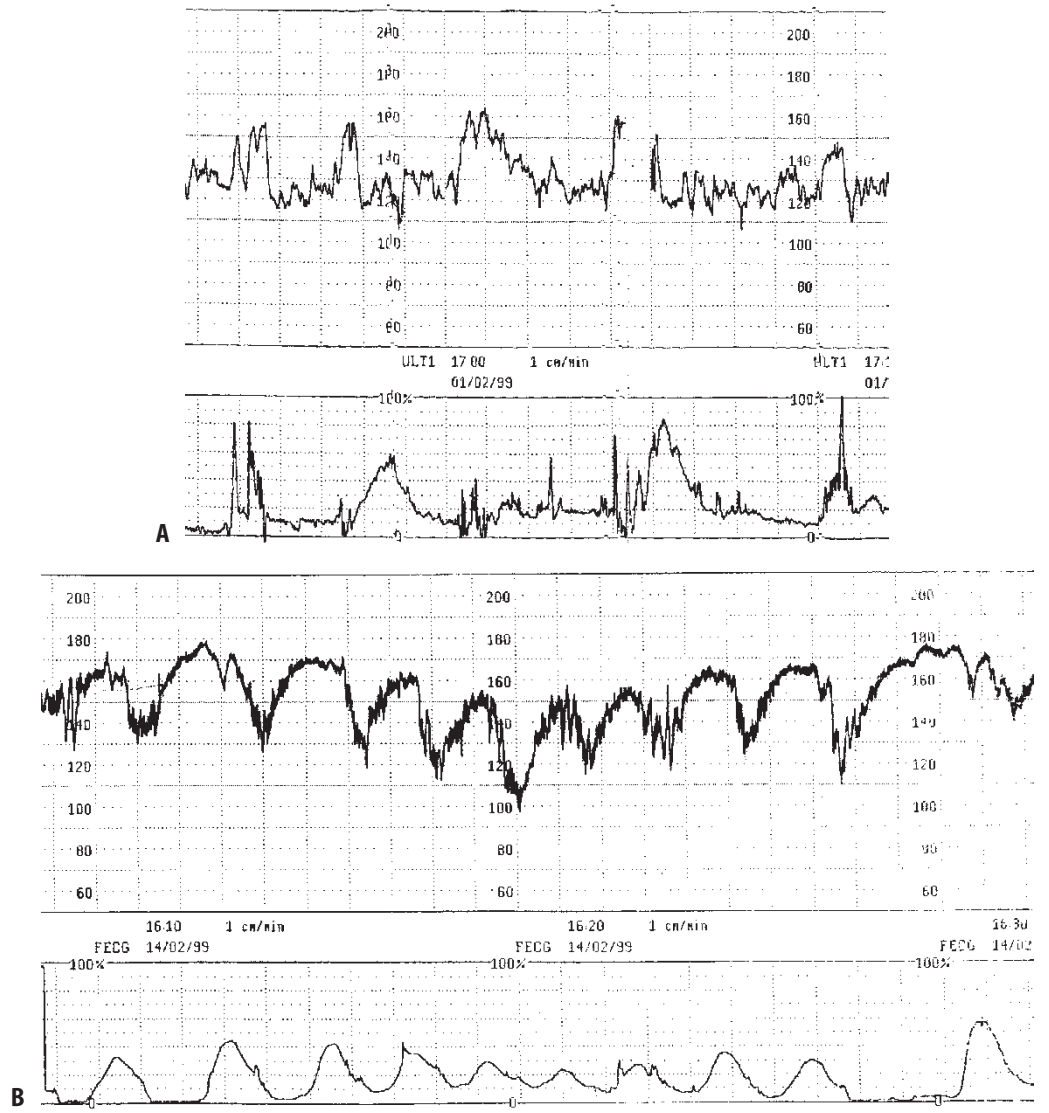

FIGURE 13.1. (A) Normal cardiotocograph (CTG) showing beat-to-beat variation and good accelerations in the heart trace. (B) Abnormal CTG shows baseline tachycardia and late (abnormal) decelerations during the second half of uterine contractions.

and death. The human fetus reacts similarly; deep, sighing respiratory movements result in squames and other amniotic debris being aspirated into terminal pulmonary air spaces.

Effects on the Fetal Brain

Intrapartum asphyxia may result in severe brain injury. Under normal circumstances autoregulation of the cerebral circulation is such that blood flow to the brain remains constant despite changes in systemic arterial pressure. However, the cerebral circulation is very sensitive to changes in paO_2 and pH, and asphyxia may result in loss of autoregulation such that cerebral blood flow does not remain constant but reflects systemic blood pressure (Greisen 1997). The hypotension that accompanies birth asphyxia is therefore associated with a high chance of cell damage in the brain, especially in watershed zones. As well as

primary cellular necrosis resulting from impaired cerebral blood flow, there is activation of apoptosis. In addition, further damage to the brain occurs after a delay of up to 48 hours as cerebral blood flow is restored—so-called secondary or reperfusion injury (Edwards et al. 1997; Fellman and Raivio 1997). There is some evidence that in long-term survivors, neurodevelopmental handicap is related to the severity of this secondary injury (Roth et al. 1992). The mechanisms of secondary injury are at present poorly understood, but free radical formation, nitric oxide synthesis, lipid peroxidation, imbalance of excitatory and inhibitory neurotransmitters, and activation of inflammatory responses all contribute to neuronal injury (Palmer 1995; Berger and Garnier 2000).

Other Effects

In the mature baby with a normal enteric nervous system, intrapartum asphyxia is commonly accompanied by passage of thick meconium into the amniotic fluid. If this is aspirated by a severely asphyxiated fetus during the gasping breathing movements that follow primary apnea, it may have serious effects on lung function after birth. Damage to the kidneys causes acute tubular necrosis; liver damage can lead to impaired production of coagulation factors (Barnett et al. 1997).

Causes

The impaired gas exchange underlying intrapartum asphyxia can result from a variety of different maternal, placental, umbilical cord, and fetal problems operating both before and during labor. Conditions that predispose to intrauterine hypoxia prior to the onset of contractions may increase the asphyxial stress during labor (Table 13.1). In addition, a number of problems are specific to parturition (Table 13.2). Significant antepartum disorders that reduce maternal, placental, or fetal reserve may first be detected during labor or may only become clinically important at this time. The well-being of the fetus depends on complex, interacting factors, and in any individual case many different variables may be operational. These include the strength and duration of uterine contractions, the distensibility of the birth canal, the size and position of the fetus, maternal oxygenation, as well as specific disorders of the maternal-placental-fetal circulations. It is not always apparent from clinical investigations or from careful examination of the baby or placenta why

Table 13.1. Major causes of antepartum hypoxia/ischemia

Inadequate gas exchange between maternal and fetal blood
Maternal disorders
Anemia
Malnutrition
Chronic renal failure
Heart disease
Peripheral arterial disease
Shock and hypoxia
Epilepsy
Aortic compression
Drugs
Preeclampsia
Pregnancy-induced cholestasis
Uterine rupture
Placental disorders
Infarction
Widespread villitis
Villous edema
Massive perivillous fibrin deposition
Abnormal villous maturation
Fetal vascular occlusion
Prolonged pregnancy
Placenta previa
Abruption
Circumvallate placenta
Inadequate gas exchange between fetal blood and fetal tissues
Cord abnormalities
Abnormal cord length
Cord entanglement
Cord knots
Cord prolapse
Cord compression
Necrotizing funisitis
Umbilical vessel thrombosis
Umbilical vessel aneurysm
Velamentous cord insertion
Fetal conditions
Fetal cardiac failure
Chronic fetal anemia
Acute fetal hemorrhage

Table 13.2. Mechanical causes of intrapartum asphyxia

Fetal malpresentation
Fetal macrosomia
Maternal pelvic abnormalities
Reduced distensibility of the birth canal
Prolonged labor
Excessive uterine contractions

intrapartum asphyxia develops in a particular case.

Maternal Disorders

Maternal problems that reduce delivery of oxygenated blood to the intervillous space increase the risk of fetal hypoxia. These include problems that predate pregnancy as well as conditions that are specific to pregnancy. Preeclampsia is the commonest of these (see Chapter 3). Severe maternal anemia and malnutrition compromise the oxygen-carrying capacity of the blood and limit the amount of oxygen that can be delivered to the placenta. The babies of mothers with chronic renal failure, heart disease, or peripheral arterial disease affecting the internal iliac or large uterine arteries are at increased risk of intrapartum stress. Maternal shock, hypoxia, grand mal fits, and compression of the aorta by the gravid uterus (Kinsella et al. 1990) can significantly reduce blood pressure and flow in the uterine arteries. Drugs administered to the mother during labor that induce hypotension, including epidural and spinal anesthetics, can have an adverse effect on uteroplacental perfusion. Abnormalities of the pelvis, uterine fibroids, and reduced distensibility of the birth canal can interfere with the descent of the fetal presenting part, with failure to progress or abnormal presentation, carry a high risk of prolonged labor and fetal asphyxia. Excessively long or strong uterine contractions, either occurring naturally or induced by oxytoxic drugs, reduce maternal blood flow to the placenta and increase the chance of fetal asphyxia even in normal-length labor.

Cholestasis of pregnancy is an uncommon disorder that is unique to the pregnant state. The development of maternal jaundice is associated with fetal asphyxia. The underlying reason for the disorder and the pathogenesis of the associated fetal hypoxia are not understood.

Uterine rupture during labor is a dire emergency leading to rapid fetal compromise with at least a 10% risk of intrapartum death [Confidential Enquiry into Stillbirths and Deaths in Infancy (CESDI) 1998]. Prior surgery, usually cesarean section, and external trauma such as motor vehicle collision predispose to uterine rupture; spontaneous rupture is less common.

Placenta Disorders

Placental disorders that reduce the volume of villous tissue available for maternoplacental gaseous exchange increase the risk of intrapartum asphyxia. Infarcts that destroy more than 10% of villous tissue, widespread villitis, massive perivillous fibrin deposition, villous edema, and abnormal villous maturation may all limit maternofetal gaseous exchange. Widespread thrombotic occlusion of fetal blood vessels significantly reduces placental reserve (Redline 1995). In a proportion of prolonged pregnancies there is diminished fetal perfusion of the villi and changes indicative of reduced uteroplacental perfusion, including stromal fibrosis, prominent syncytial knots, and reduced numbers of vasculosyncytial membranes (Fox 1997). Such changes may predispose to hypoxia during labor.

Some placental disorders, including complications of placenta previa and acute retroplacental hemorrhage, often result in obstetric emergencies. In placenta previa, implantation is abnormally low in the uterus. As the lower uterine segment is taken up during the second half of pregnancy, there may be premature separation of the low-lying part of the placenta. The resulting retroplacental hemorrhage often happens before term; thus it is a more common cause of intrauterine hypoxia in preterm than in full-term infants. Acute retroplacental hemorrhage with a normally sited placenta is associated with spiral artery abnormalities in the placental bed, and is a complication of pregnancy-induced hypertension (Dommisse and Tiltman 1992). Its occurrence is unpredictable, and a major abruption during labor can cause rapid fetal asphyxia and death. A circumvallate placenta, especially associated with hemosiderin deposition in the membranes and a history of vaginal bleeding in the first and second trimester, is held by some to be a reflection of intrauterine ischemia preceding labor. Once vaginal bleeding ceases, the pregnancy may appear normal until the infant develops intrapartum asphyxia and may subsequently manifest signs of brain injury (Redline 1995).

Umbilical Cord

The umbilical cord is the fetal lifeline, and any factor that compromises flow through the umbili-

cal vessels has severe consequences for the infant. Abnormally short cords are subject to excessive traction as the fetal body descends through the birth canal. Tension results in spasm of the umbilical cord vessels and a sharp reduction in fetoplacental blood flow. Short cords also carry a risk of premature placental separation; those measuring less than 30 cm at term are incompatible with a normal vaginal delivery (Fox 1997). Excessively long cords carry an increased risk of entanglement around the fetal body or limbs and are more likely to have true knots or excessive coiling than cords of normal length. Baergen et al. (2001) found an increase in neurologic abnormality in babies with long cords. Nuchal cords wrapped once around the neck are relatively common. They are found in 29% of deliveries by 42 weeks' gestation (Larson et al. 1997). They do not constitute a risk to the fetus (Gonzales-Quintero et al. 2004). Multiple nuchal encirclement was associated with the passage of meconium and fetal heart rate abnormality in late labor without adverse fetal outcome (Larson et al. 1995). Complex limb encirclement can lead to hypoxic insult. Fetal movement, including descent of the presenting part during labor, can result in traction on an entangled cord or tightening of a knot with reduction in fetoplacental blood flow. A significant cord knot will show differential congestion on either side of the knot (Fig. 13.2).

Another obstetrical emergency is prolapse of the umbilical cord into the vagina. In this position, there is a risk of cord compression by the presenting fetal part, with sudden obstruction to fetoplacental blood flow. Cord prolapse in more likely in preterm labor and multiple pregnancy. One study found that many deaths were related to extreme prematurity or lethal malformations (Murphy and MacKenzie 1995). Cord compression also occurs in term pregnancies complicated by oligohydramnios.

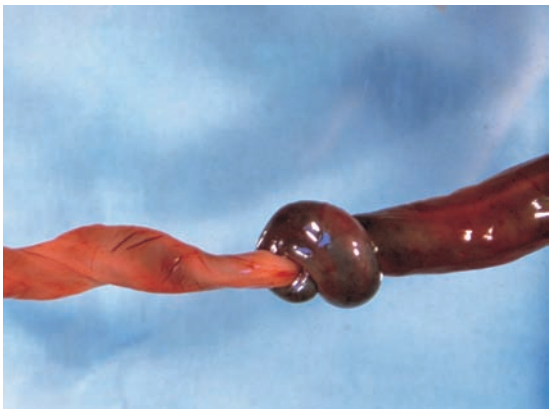

Figure 13.2. Term stillbirth at 39 weeks' gestation. There is a tight true knot in the umbilical cord with congestion on one side and pallor and loss of turgor on the other.

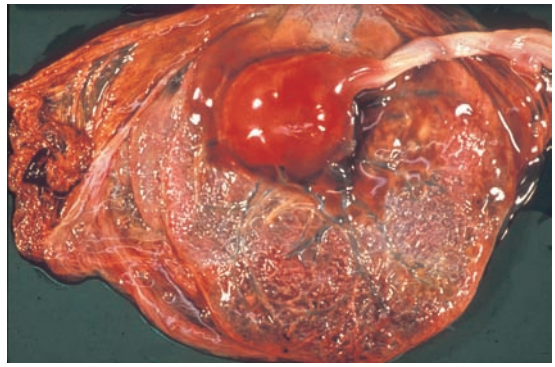

Figure 13.3. Cord insertion into the placenta. There is a tense aneurysm in an artery at the point of insertion.

Intrinsic umbilical cord pathology, such as necrotizing funisitis, vascular thrombosis, or aneurysm may also reduce flow through the umbilical vessels (Fig. 13.3). Even if there is no overt fetal asphyxia prior to the onset of contractions, such abnormalities accentuate the hypoxic stress of labor and result in severe intrapartum asphyxia. Cords with velamentous insertion have unprotected vessels running in the membranes that are at risk of compression, thrombosis, and rupture, especially when there is vasa previa, the last resulting in rapid fetal exsanguination (Fig. 13.4).

Fetal Conditions

Certain fetal problems may interfere with oxygen delivery during labor and result in intrapartum asphyxia. Prior to the onset of labor, provided there is no cord compression, perhaps as a result of oligohydramnios, the position of the fetus is not a major factor in oxygen delivery. However, this changes once labor starts. A fetus presenting by the vertex inferior with a well-flexed head and the occiput anterior presents the smallest cranial diameter to the birth canal and is the optimum

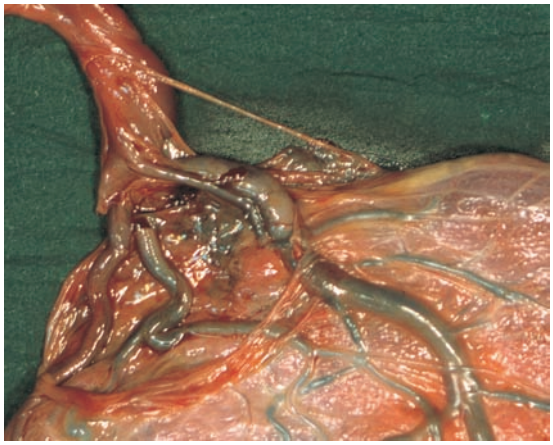

Figure 13.4. Placenta from a growth-restricted fetus at 35 weeks, with velamentous insertion of the umbilical cord complicated by thrombosis of a velamentous vein. A band of amnion runs between the cord surface and the chorionic plate.

position for delivery. Any malpresentation, or incomplete flexion or failure of rotation of the head during descent through the birth canal increases the risk of long or obstructed labor and of intrapartum asphyxia. Malpresentation also increases the likelihood of cord prolapse. Breech presentation raises the possibility that the trunk will pass through an incompletely dilated cervix with subsequent delay to delivery of the head. Breech presentation that is not diagnosed before the onset of labor is a particular hazard (CESDI 2000). The risk of asphyxial insult resulting in death or neurological abnormality is three times higher in breech presentation following planned vaginal delivery at term than after elective operative delivery (Herbst and Thorngren-Jerneck 2001; Hofmeyr and Hannah 2003; Rietberg et al. 2003). Fetal factors that increase the chance of breech presentation include intrauterine growth restriction, multiple pregnancy, hydrocephalus, and neuromuscular disorders. Poly- and oligohydramnios also increase the risk. Malpresentation occurs more commonly in preterm labors than in those after 34 weeks, because of the relatively large amount of liquor amnii at that time. Incomplete flexion or failure of rotation of the head during delivery are seen in fetuses with neuromuscular abnormalities or malformations of the cervical spine, and in those with head and neck tumors.

Fetal macrosomia increases the risk of difficult delivery, often with shoulder dystocia or hypoxic insult. The risk of intrapartum death of babies ⩾4000 g is twice that of babies weighing 2500 g to 3999 g (CESDI 1999). There is an increased risk of uterine rupture.

Fetal cardiac failure from any cause and chronic fetal anemia from red cell loss or destruction increase the risk of fetal asphyxia during labor. Acute fetal blood loss during parturition is uncommon but devastating. The usual causes in singletons are fetomaternal hemorrhage or rupture of vasa previa from velamentous insertion of the umbilical cord (Fig. 13.5). The latter can cause exsanguination of the fetus within minutes; diagnosis is often delayed because of confusion with placental abruption. With monochorionic twins, acute twin-to-twin transfusion can occur during delivery with major blood loss from the donor infant.

Pathological Findings

Early Deaths

The major pathological findings in asphyxiated infants who die during or shortly after labor are listed in Table 13.3. Specific organ damage is not usual in early deaths, as both gross and microscopical features of asphyxial lesions take time to develop.

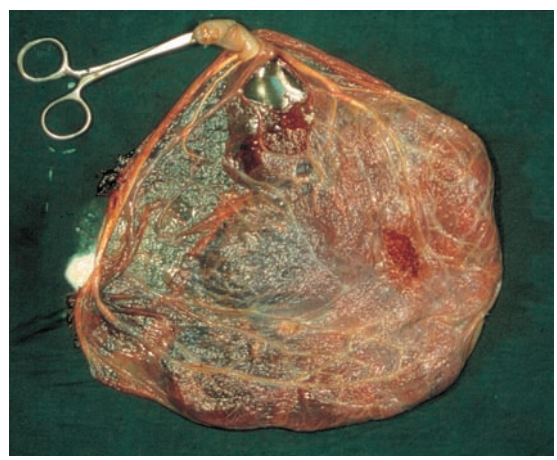

Figure 13.5. Placenta from a 31-week stillbirth following membrane rupture and vaginal bleeding. There is vasa previa, white barium/gelatin spills from a ruptured vein.

Table 13.3. Pathological findings in early asphyxial deaths

Meconium staining of skin
Meconium in larynx/trachea
Lung, epicardial and thymic petechial hemorrhages
Aspirated squames ± meconium in air spaces
Meconium in the stomach
Congestion and edema in the brain
Renal cortical or corticomedullary hemorrhage
Adrenal hemorrhagic necrosis

Meconium staining of the skin may be evident. When the body has been washed, this may be apparent only on careful examination of the fingernails, behind the ears, between the toes, and in skin creases. Cutaneous petechial hemorrhages are sometimes visible, usually over the presenting part or the face, neck, and chest (Fig. 13.6), particularly following shoulder dystocia or placental abruption. These should not be confused with the widespread hemorrhages typical of some fetal infections. There is generally deep purple discoloration of the skin and nails. Pallor may be apparent following acute hemorrhage. Skin slipping or other evidence of maceration is unusual, except in the presence of fetal hydrops. External examination may suggest the reason for asphyxia, for example evidence of growth restriction, macrosomia, hydrops, or fixed flexion deformities due to neuromuscular abnormalities (Fig. 13.7) or prolonged oligohydramnios.

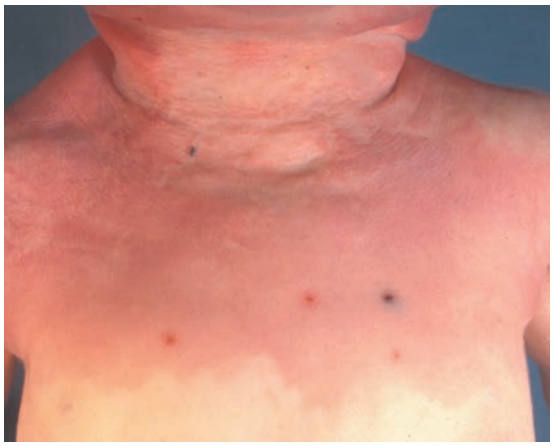

Figure 13.6. Fresh stillbirth at 42 weeks, birth weight 4900 g. There was shoulder dystocia; petechial hemorrhages are present on the front of the chest.

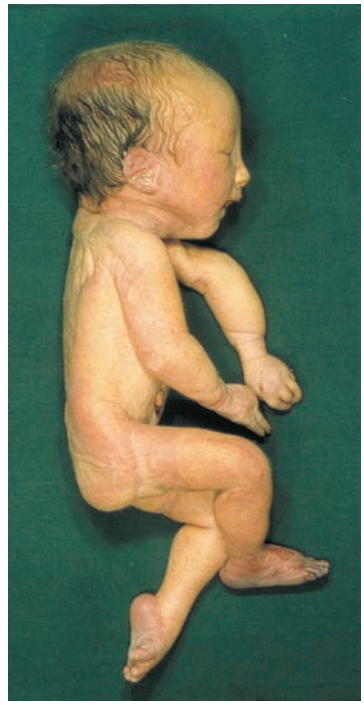

Figure 13.7. Neonatal death at 2 days, 35 weeks' gestation. Flexion deformities are a manifestation of congenital muscular dystrophy.

Internal examination, unless associated with fetal anemia or acute hemorrhage, reveals intense congestion of organs and tissues. Petechial hemorrhages are commonly present beneath the visceral pleura, on the epicardial surface of the heart, especially around the coronary vessels and in the thymus, although they are not seen in every case. Petechial hemorrhages are particularly large and numerous after placental abruption. A large retroplacental hemorrhage compresses the villous tissue, increasing both fetal blood volume and pressure (Wigglesworth 1998). Abnormal coagulation is common in asphyxiated babies and may exacerbate any bleeding tendency.

The lungs of babies who are stillborn or die shortly after birth are generally solid and dark red with focal subpleural hemorrhages. Patchy distention of air spaces, interstitial emphysema, or subpleural blebs may reflect attempted resuscitation. There may be meconium staining of the larynx and meconium-stained material in the bronchi. Massive meconium aspiration into the lungs is sometimes detectable on macroscopic

examination, the cut surface of the lung having a greenish tinge. Ingested meconium may be present in the stomach.

The brain usually shows little gross abnormality, changes being limited to vascular congestion and mild edema. Hemorrhage into the falx and small subarachnoid hemorrhages over the cerebral convexities are common. On slicing the brain, there may be some compression of the lateral ventricles. In preterm infants, germinal matrix and intraventricular hemorrhage are most commonly seen in those who survive for a day or more, but may be apparent in those who die before 12 hours of age or are stillborn.

Adrenal hemorrhage and necrosis are common and may be massive (Fig. 13.8), especially after breech delivery. Adrenal hemorrhage is not specific for asphyxia and complicates intrauterine infections such as bacterial septicemia and herpes virus infection. Hemorrhage may be seen in other organs, especially in the kidneys and in the testis, although it may only be apparent on histological examination.

The limited and nonspecific nature of pathological findings in infants who die shortly after sustaining a lethal asphyxial insult means that histological examination of major organs and placenta is mandatory. Histological examination of the lungs from babies who are stillborn or die in the early neonatal period confirms or excludes the presence of intrauterine infection.

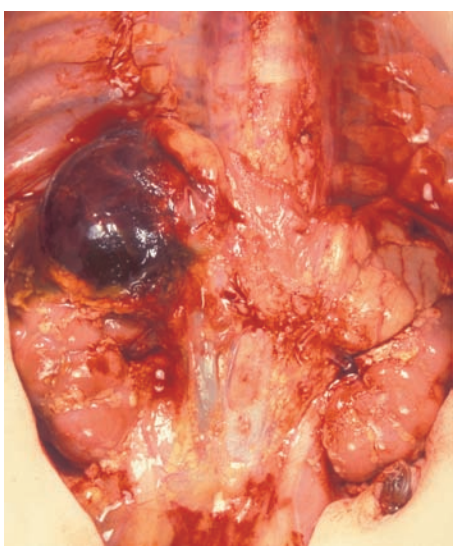

Figure 13.8. Mature baby, death at 4 days, intrapartum hypoxic stress. There is a massive right-sided adrenal hemorrhage.

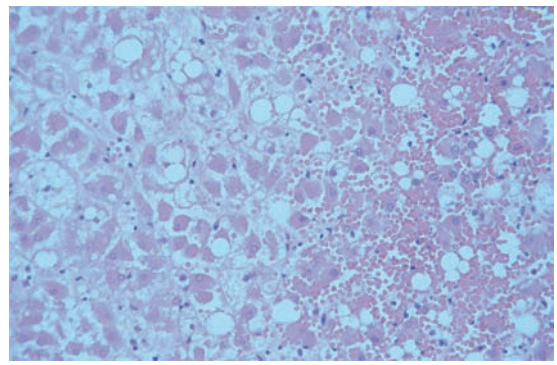

Figure 13.9. Intrapartum death at term, placental abruption. The adrenal cortex displays marked congestion and early fatty change.

While the severity of myocardial damage determines immediate postnatal survival, histological abnormalities in the myocardium of infants who die during labor are usually nonspecific and do not correlate well with clinical impressions of myocardial dysfunction.

The brain commonly shows only white matter edema, unless there was antepartum anoxic-ischemic brain injury. Neuronal or white matter necrosis will not be identified in a term infant who has not survived for at least 18 to 24 hours from the time of insult. Detailed histological examination of the fixed brain is essential to identify evidence of hypoxic-ischemic brain injury that preceded the onset of labor.

Acute stress changes, in particular a starry sky pattern in the thymic cortex, are likely in all early neonatal deaths. However, after intrapartum death, the presence of significant thymic cortical lymphocyte depletion and fatty change in the fetal cortex of the adrenal glands indicates fetal compromise prior to the onset of labor (Fig. 13.9). Increased numbers of circulating nucleated hemopoietic cells, in the absence of fetal anemia, are thought to be an indication of antemortem hypoxia (Fig. 13.10) (Korst et al. 1996).

Late Deaths

In infants who survive for some days after an acute intrapartum asphyxial episode, the findings are

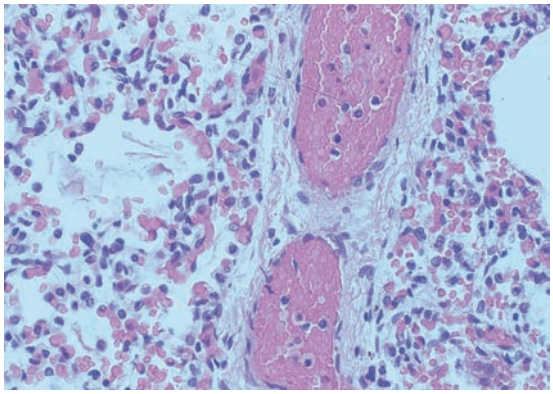

Figure 13.10. Term infant, death at 6 hours after intrapartum hypoxia. Within the congested lung, squames are present in air spaces; an excess of circulating nucleated cells indicates antepartum hypoxia.

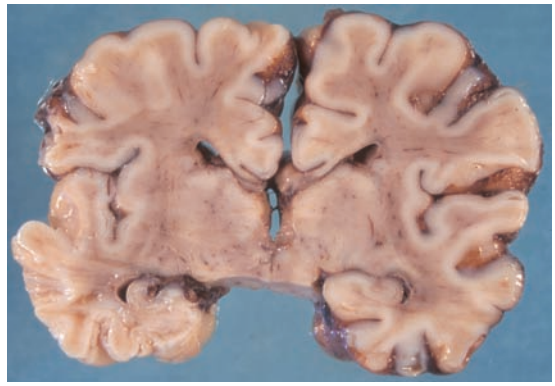

Figure 13.11. Term infant, died at 3 days, severe hypoxic stress. The cerebral cortex has a pale cortical ribbon that contrasts with the congested, violaceous white matter.

different. The intense congestion typically seen in the immediate post-asphyxial period has generally resolved. Meconium staining of the skin and respiratory tract is no longer visible and there is no meconium present in the gastric lumen. Petechial hemorrhages over the thoracic viscera have generally dispersed, although adrenal hemorrhagic necrosis may still be apparent with evidence of organization. Abnormalities in other organs may be present at a histological level (Table 13.4). The pulmonary sequelae are described in Chapter 20.

There is often evidence of raised intracranial pressure with a bulging anterior fontanelle and separation of the sutures (see Fig. 2.22 in Chapter 2). The brain is swollen with flattened convolutions, and cerebellar tonsillar herniation is occasionally seen. On slicing the brain, the pallor of the cortical ribbon is distinct from the congested subcortical white matter (Fig. 13.11). Patterns of brain damage that are seen after intrapartum asphyxia become increasingly evident with length of survival. The pattern of damage is gestation-related and influenced by the severity and duration of asphyxia. These changes may, in the future, be modified by treatment designed to reduce the impact of secondary "reperfusion" brain injury. Estimating the age of any damage is important in distinguishing antepartum from intrapartum brain injury. This becomes increasingly difficult with increasing length of postnatal survival (Becher et al. 2004), particularly after 3 days of age. Patterns of cerebral injury associated with intrapartum injury are summarized in Table 13.5 (Perlman 1997). More than one type of lesion may be found.

In mature infants who have sustained asphyxial brain injury, bilateral damage to the cortical gray matter and subjacent white matter is the commonest finding. This occurs particularly in

Table 13.4. Pathological findings in delayed death after intrapartum asphyxia

Lungs:	aspirated squames ± meconium, hyaline membranes, constricted, and muscularized pulmonary arterioles, pneumonia
Brain:	generalized swelling, flattening of the gyri, varying patterns of neuronal and white matter necrosis
Heart:	focal papillary muscle necrosis
Kidneys:	acute tubular necrosis, massive parenchymal hemorrhage (uncommon), medullary necrosis (uncommon)
Liver:	fatty change, perivenular necrosis
Intestines:	necrotizing enterocolitis

Table 13.5. Patterns of hypoxic-ischemic cerebral injury after intrapartum asphyxia

Parasagittal injury to cerebral cortical gray matter and subjacent white matter
Damage to the deep grey matter
Multifocal ischemic infarction
Periventricular leukomalacia
Selective neuronal necrosis
Global necrosis (following cardiac arrest)

Source: Perlman (1997).

parasagittal areas, neuronal damage often being greatest in the depth of sulci with relative sparing of gyral crests. Histology reveals neuronal apoptosis and necrosis, with a variable cellular reaction that is time dependent. After several weeks, there is shrinkage and gliosis of the affected cortex, which becomes thin and scarred, with cystic change in the subcortical white matter if damage has been severe (Fig. 13.12). A less common pattern of damage is necrosis predominantly affecting the neurons in the deep gray matter, especially the thalamus, caudate nucleus, globus pallidus, and putamen. Sequential changes are described in Chapter 26. In preterms, hemorrhagic infarction of cortical white matter can be extensive (Fig. 13.13), or multifocal in watershed areas. Periventricular leukomalacia occurs adjacent to the external angles of the lateral ventricles, and is commonest before 34 weeks' gestation, when this is a watershed area and thus sensitive to reduced cerebral perfusion. Ultimately, if the foci of necrosis are large, they may undergo cystic change and there may be residual ventricular dilatation (Fig. 13.14).

In the heart, focal necrosis is seen in the inner third of the myocardium, particularly in the papillary muscles (Barnett et al. 1997) (Fig. 13.15); its demonstration may require examination of multiple blocks, including longitudinal blocks through the papillary muscles (Donnelly and Hawkins 1987). Histological changes are frequently not as impressive as would be expected from the degree of impairment of left ventricular contractility noted clinically.

Renal failure is common in infants who survive intrapartum asphyxia, but at necropsy histological changes are not impressive. The coexistence of hemorrhage, eosinophilic secretions within

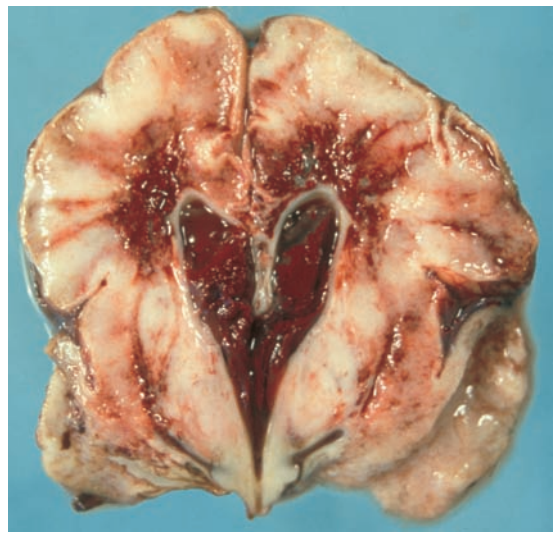

Figure 13.13. Preterm, died at 1 day. There is extensive hemorrhagic infarction of the cortical white matter and bilateral intraventricular hemorrhage.

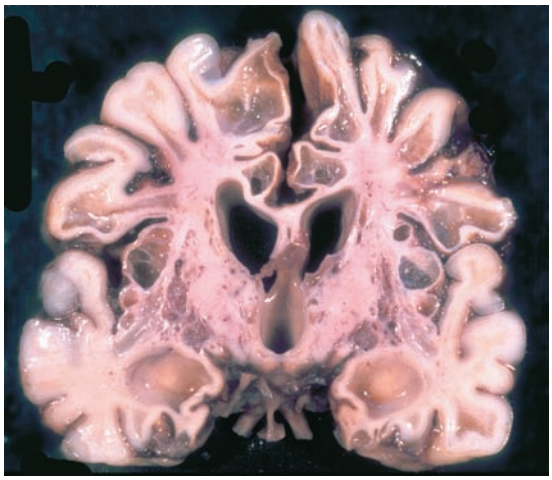

Figure 13.12. Term infant, intrapartum hypoxia, died at 37 days. There is extensive cystic degeneration of the cortical white matter.

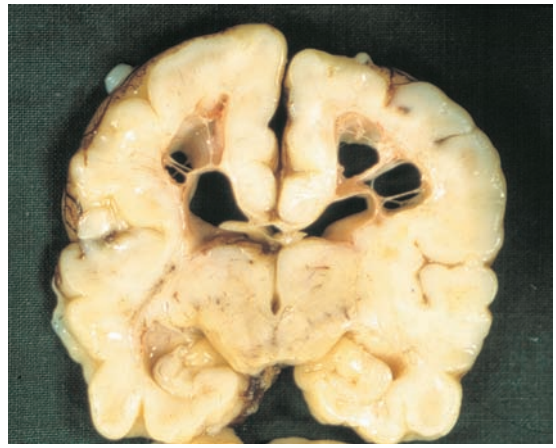

Figure 13.14. Preterm birth at 25 weeks, died at 8 weeks. Coronal slice of fixed brain contains large cysts in the periventricular white matter, which are the result of perinatal white matter infarction.

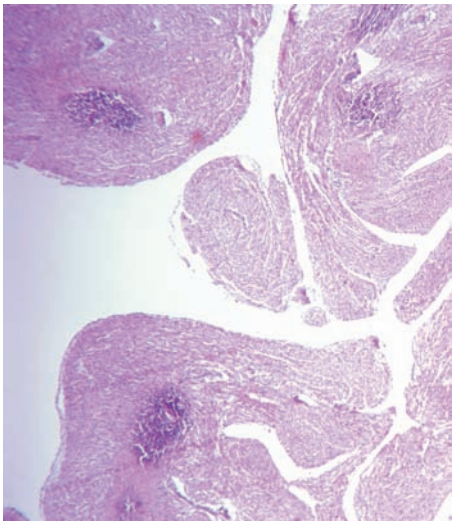

Figure 13.15. Calcification in foci of necrosis within cardiac papillary muscles following hypoxic/ischemic insult.

tubules, and mitotic figures in tubular epithelium help to distinguish antemortem pathology from postmortem artifact. Occasionally, there is massive renal cortical hemorrhage or hemorrhagic infarction is seen.

In the liver, marked microvesicular fatty change may develop in hepatocytes. However, if fatty change is more marked in the right than the left lobe, it is a reflection of antepartum stress. Periportal hemorrhage and necrosis of hepatocytes around portal veins is common (Barnett et al. 1997).

Severe birth asphyxia is associated with constriction of the vessels of the splanchnic circulation; necrotizing enterocolitis may result, particularly in term infants.

Birth Trauma

Definition and Incidence

Birth trauma comprises physical injuries sustained by the fetus as a consequence of the birth process. Identification of traumatic lesions does not confer significance in respect of causation of death. The contribution of any putative lesion requires careful consideration. The presence of some relatively trivial lesions, such as shearing tears of the tentorium, should alert the prosector to the possibility of a perhaps unsuspected intrapartum problem and the probability of asphyxial insult. It is not as rare as many wish to believe. A Finnish study of 14,265 live births published in 1990 found major trauma in 3.16% of the infants (Salonen and Uusitalo 1990). However, the most common lesions such as Erb's palsy and fractured clavicle, are not life threatening and consequently are infrequently encountered by pathologists. They occur most often following a prolonged second stage of labor, shoulder dystocia, instrumental delivery, and macrosomia (Perlow et al. 1996; Oral et al. 2001; Raio et al. 2003). Severe birth trauma resulting in intrapartum or early neonatal death is infrequent in countries where there is good obstetric care. A U.K. study identified major craniocervical trauma in only 0.031/1000 total births (37/1,191,299 births) in babies >2500 g, or 37/709 (5%) of intrapartum–related deaths in that birth weight group (O'Mahoney et al. 2005). Cases were identified clinically and 30% had no necropsy findings. Only 57% of perinatal deaths came to necropsy during the study period, so the derived rate of injury is a minimum. Nevertheless, a regional perinatal pathology department in the U.K. investigating 450 perinatal deaths per year might expect to examine two infants each year with significant cranial birth trauma.

Pathophysiology and Causes

Birth trauma is closely linked with intrapartum asphyxia. Many of the conditions that predispose to intrapartum asphyxia also directly increase the risk of trauma during delivery (Table 13.6). Moreover, birth trauma resulting in shock and hypotension increases the severity of hypoxic-ischemic damage to the brain and other organs.

Table 13.6. Factors predisposing to birth injury

Asphyxia
Instrumental delivery
Malpresentation
Obstructed or prolonged labor
Fetopelvic disproportion
Macrosomia
Fetal abnormality
Epidural anesthesia

The greatest risk of birth trauma is instrumental delivery (Ruggieri et al. 1999; Towner et al. 1999; O'Mahoney et al. 2005). Such intervention is more likely when there is clinical evidence of fetal hypoxia. The organ congestion and poor muscle tone in asphyxiated infants increases the risk of injury during delivery. However, birth trauma is not confined to instrumental deliveries. Injury can occur during cesarean section or unassisted vaginal delivery (Vasa and Kim 1990; Heise et al. 1996; Durham et al. 1998). The relative contributions of asphyxia and trauma to the death of an individual infant may be difficult to assess. Careful note of the clinical history and antemortem findings is essential, and information about the oxygen concentration and pH in cord blood should be available to the pathologist. If the cord gases are borderline and necropsy reveals an injury with major bleeding but no evidence of anoxic-ischemic injury, and the postnatal course of the infant is in keeping with shock from blood loss, birth trauma is the likely cause of death. However, in many cases, it may not be possible to determine whether the asphyxia or trauma is the more important condition (CESDI 1998). Under these circumstances the pathologist may have to give intrapartum asphyxia and trauma as the cause of death without attempting to prioritize one over the other.

The fetus with multiple contractures (arthrogryposis) is at risk of injury during vaginal delivery as is the growth restricted, malnourished fetus with a thin skull, in whom there is an increased risk of excessive cranial distortion and significant subdural hemorrhage during delivery. Clinical problems are often ascribed to asphyxia, birth trauma being diagnosed only during postmortem examination.

Death from birth trauma is most likely when the brain or spinal cord is damaged. The fetal head is malleable and designed to withstand uniform compression during delivery. Incorrect application of forceps to the fetal head may result in an asymmetrical force being transmitted to intracranial structures, with a risk of tearing of dural folds and blood vessels. The use of vacuum extraction may cause an excessive shearing force to a localized area of the head, a situation that may result in tearing of the blood vessels, producing a significant subaponeurotic hemorrhage. Vacuum extraction has also been associated with tearing of intracranial structures. During a difficult vaginal breech delivery there is a risk of damage to the spinal cord if excessive traction is placed upon the neck. The use of rotational forceps is associated with several types of trauma including high spinal cord injury. Damage to the spinal cord during instrumental delivery may be secondary to ischemia if the vertebral arteries are damaged.

Types of Injury

Physical injury to almost any organ or tissue can occur during delivery. Literature reviews describe the injuries encountered (MacKinnon et al. 1993; Perlow et al. 1996; Medlock and Hanigan 1997), many of which are unlikely to be seen at autopsy. Birth injuries likely to be encountered by the pathologist are listed in Table 13.7, and sites of pericranial and intracranial hemorrhage are shown in Figure 13.16.

Laceration and Bruising

Cutaneous lacerations occur occasionally during cesarean section and are usually of no major importance. Scalp laceration (Teng and Sayre 1997) and abrasions (Ross et al. 2000) can complicate Ventouse application.

TABLE 13.7. Major birth injuries

Major injuries	Risk factors
Bruising and lacerations	Malpresentation, forceps delivery
Caput succedaneum	Normal vaginal delivery, vacuum extraction
Subaponeurotic (subgaleal) hemorrhage	Vacuum extraction, forceps, rarely SVD
Subperiosteal hemorrhage (cephalohematoma)	SVD, skull fracture
Extradural hemorrhage	Skull fracture
Subdural hemorrhage	Forceps, precipitate delivery, fractures, occipital osteodiastasis
Tears of dural folds	
Skull fractures	Forceps; rarely cesarean section or SVD
Occipital osteodiastasis	Vaginal breech delivery; rotational forceps
Spinal cord injury	Vaginal breech delivery, rotational forceps
Visceral injuries and long bone fractures	Presentation-related

SVD, spontaneous vertex delivery.

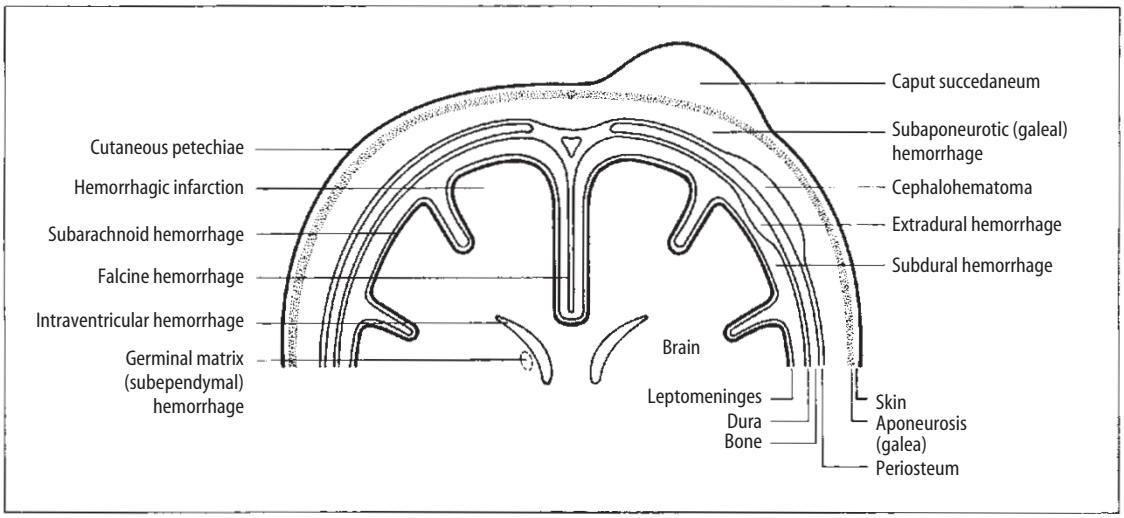

Figure 13.16. Sites of asphyxial (left side) and traumatic (right side) pericranial and intracranial hemorrhage.

Bruising is common, especially in preterm infants, after instrumental delivery and with malpresentation. Following forceps delivery, the position of the blades can often be identified by bruising and excoriation of the skin (Johnson et al. 2004) (Fig. 13.17). Superficial bruising usually has no significant adverse clinical effect, although application of forceps over the eye or facial nerve may have serious sequelae. Deep bruising is more common and extensive after preterm delivery, with hemorrhage into muscles of the limbs and buttocks (Figs. 13.18 and 13.19). Blood loss may result in hypovolemia and subsequently jaundice, as the red cells break down; the extent of bleeding may not always be apparent from inspection of the skin. Incision into underlying muscle will give a better appreciation of the extent of hemorrhage.

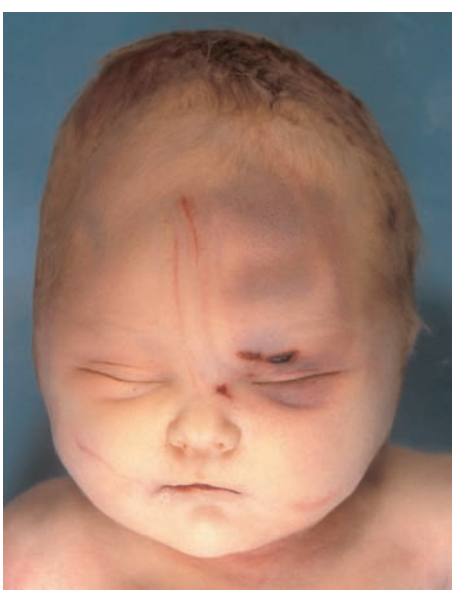

Figure 13.17. Term stillbirth, forceps delivery for fetal distress. There is frontal and intraorbital bruising. The mark of the forceps blade is visible across and above the eye.

Caput Succedaneum and Chinon

Compression of the presenting part against the internal os results in venous stasis with swelling and bruising and is normal after vaginal delivery. During vertex presentation, edema fluid and blood collects in the scalp superficial to the muscular aponeurosis. This forms the so-called caput succedaneum, which usually resolves rapidly after delivery and is of no clinical significance. Following vacuum extraction, a more circumscribed areas of swelling known as a "chinon" forms beneath the site of cup application. Bleeding may be considerable and damage to the skin is not uncommon, resulting in scarring and baldness.

Subaponeurotic (Subgaleal) Hemorrhage

Damage to scalp vessels by shearing stress during delivery can result in massive bleeding into the

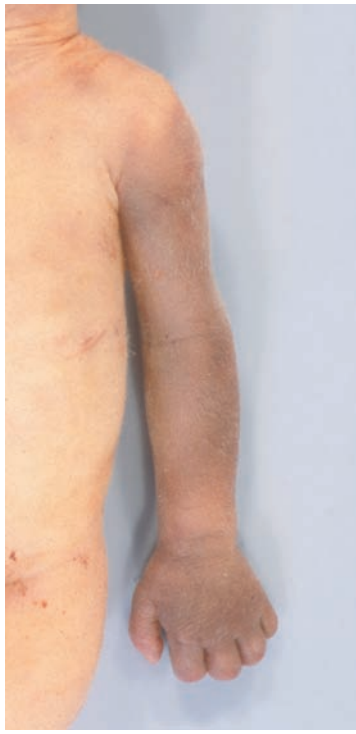

Figure 13.18. Second twin, shoulder presentation. There is bruising of the whole arm, which prolapsed through the cervix.

tissue plane between the deep aspect of the aponeurosis of the occipitofrontalis muscle and the periosteum on the outer surface of the skull. This muscle is only firmly attached to the skull posteriorly and over the zygomatic arches. Hemorrhage into the subaponeurotic space can extend all over the skull surface and into the subcutaneous tissues of the neck without restriction; the scalp feels boggy and the head circumference is increased (Fig. 13.20). Blood loss may be massive and is exacerbated by consumption coagulopathy (Kilani and Wetmore 2006). It can occasionally result in compression of the underlying brain (Amar et al. 2003). Robinson and Rossiter (1968) calculated blood losses in excess of 100 mL, which is between one third and one half of circulating blood volume in a term neonate. The resulting hypovolemia and shock may be confused clinically with birth asphyxia, the significance of hemorrhage being apparent only at necropsy. Associated injuries are common; one third with subdural hemorrhage and 19% with skull fracture were found in one center (Kilani and Wetmore 2006).

The major risk factor for subaponeurotic hemorrhage is Ventouse extraction, particularly when multiple pulls, reapplication of the cup, recourse to forceps application, or emergency cesarian section are required (Chadwick et al. 1996; Gebremariam 1999; Boo et al. 2005; O'Mahoney et al. 2005). Application of the cup over the sagittal suture or close to the anterior fontanelle increases the risk of subgaleal hemorrhage (Boo et al. 2005).

Subperiosteal Hemorrhage (Cephalhematoma)

Shearing stress can elevate the periosteum from the outer table of the skull during delivery, resulting in bleeding between the periosteum and the bone. Reflection of the scalp reveals a circumscribed, dome-shaped hematoma limited by attachment of the periosteum at suture lines, overlying a single bone (Fig. 13.21), although separate hematomata over different bones can coexist. Subperiosteal bleeding occurs after instrumental delivery and in association with skull fractures, but is seen most commonly after spontaneous vaginal delivery. It does not, on its own, result in sufficient blood loss to produce shock, but a lump

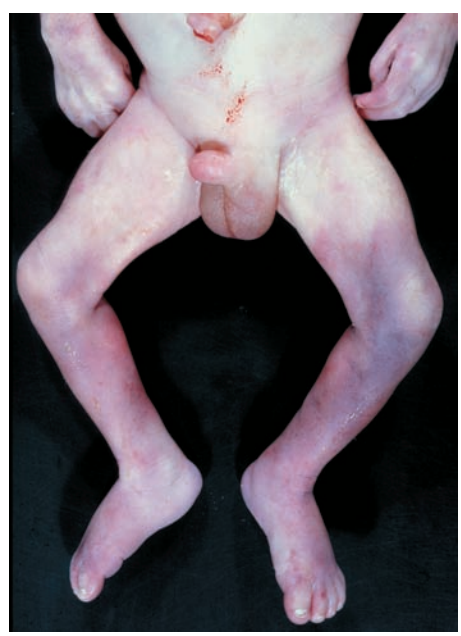

Figure 13.19. Breech presentation, preterm delivery. There is extensive bruising of both legs.

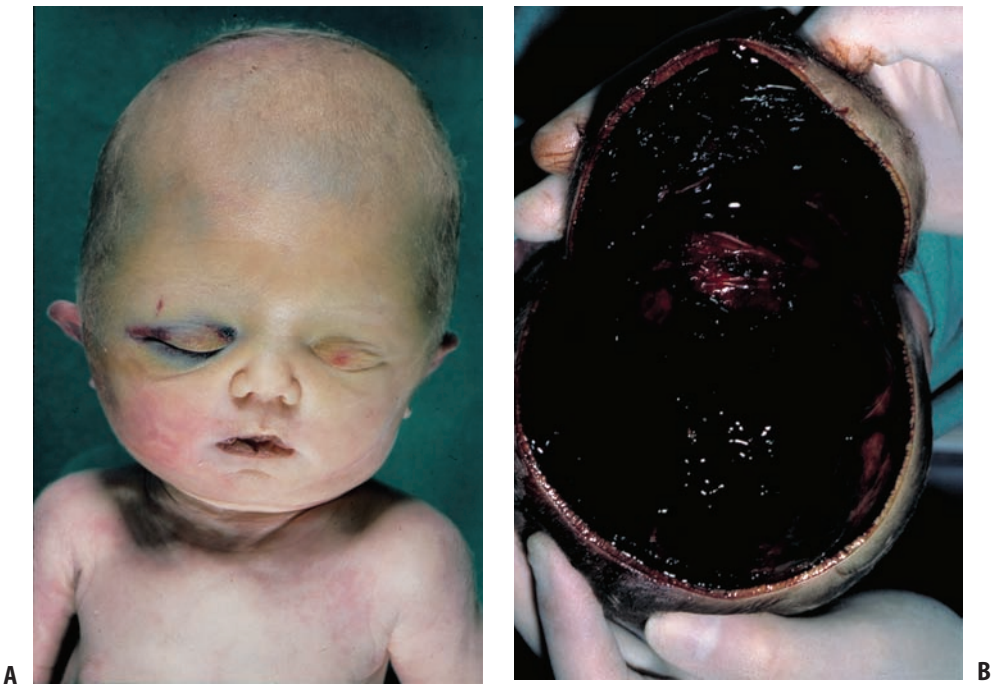

Figure 13.20. (A) Scalp swelling and periorbital bruising due to subaponeurotic hemorrhage. (B) Reflection of the scalp reveals massive subaponeurotic hemorrhage. Delivery by vacuum extraction. (Courtesy of Dr. A.G. Howatson, Glasgow.)

may be observed on the baby's head. As the hematoma resorbs, bone is laid down beneath the elevated periosteum, resulting in a hard, smooth lump that may give rise to concern about the possibility of nonaccidental injury (Rupp et al. 2005) (Figs. 13.22 and 13.23). Subsequently, remodeling occurs until the normal contour of the head is restored.

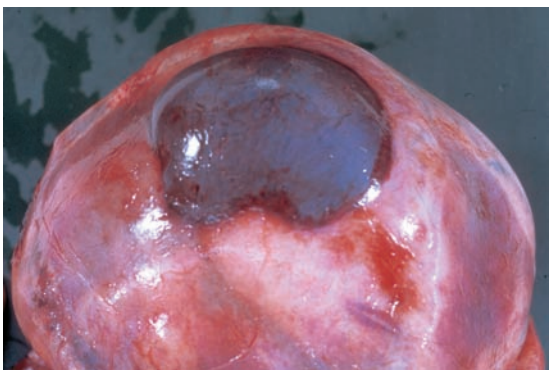

Figure 13.21. Fresh subperiosteal hematoma (cephalhematoma) overlying the right parietal bone.

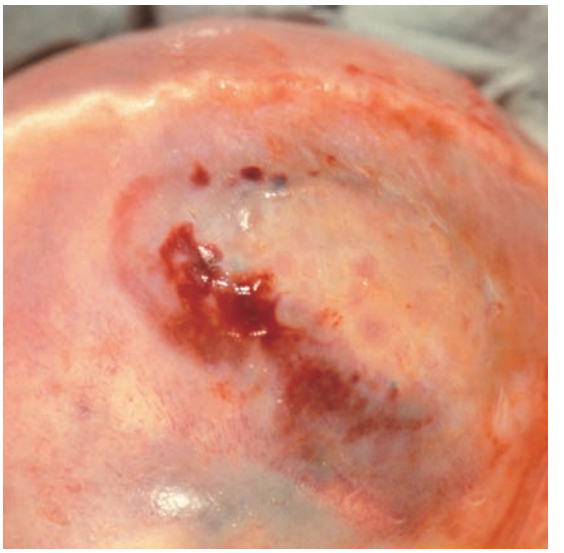

Figure 13.22. Sudden infant death at 8 weeks. An organizing parietal cephalhematoma gave rise to suspicion of inflicted injury.

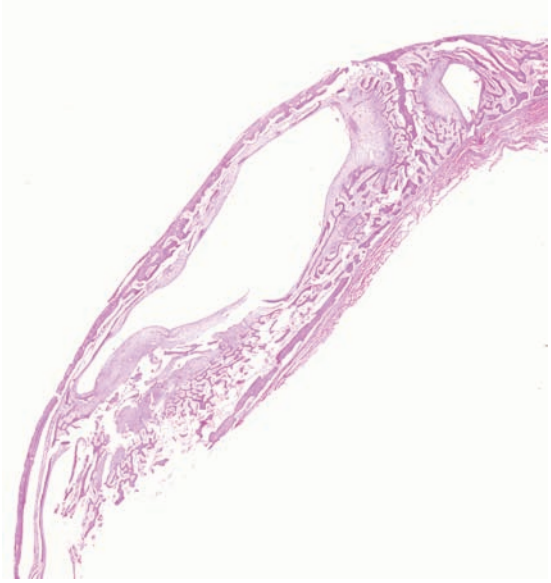

Figure 13.23. Section through an organizing cephalhematoma. New bone is present beneath the periosteum. The original line of the parietal bone is the right margin of the section.

Extradural Hemorrhage

In infants, the dura is firmly attached to the inner table of the skull, and extradural hemorrhage is unusual, other than a thin film of blood along fracture lines.

Subdural Hemorrhage

Minor subdural hemorrhage (SDH) is a common manifestation of birth trauma. The usual cause is rupture of bridging veins as they run across the subdural space, resulting in a thin film of blood or a small blood clot on the surface of the brain. Small SDHs have been demonstrated in 8% of liveborn infants in a small study using magnetic resonance imaging (Whitby et al. 2004). While they are more common following instrumental delivery, they were seen in 6% of babies following normal vaginal delivery. Rupture of bridging veins follows rapid or asymmetric changes to the shape of the cranium, and is most common after instrumental or precipitate delivery. Fronto-occipital compression of the head causes kinking of and obstruction to the vein of Galen. Occasionally, this vessel ruptures, causing massive SDH. Damage to the venous sinuses, either secondary to a dural tear that extends into a sinus or laceration of a sinus by the edge of a fractured or dislocated skull bone, is another cause of large SDH. It is uncommon where there is good obstetric care. An SDH can complicate hemorrhagic infarction of the underlying brain (Steinbok et al. 1995) in the absence of trauma.

The effects of SDHs vary with the size of the bleed, its site, and its cause. Relatively small bleeds in the posterior fossa can cause brainstem compression and respiratory arrest. Prompt diagnosis and evacuation may be lifesaving (Perrin et al. 1997). Small bleeds over the cerebral hemispheres may not cause neurological signs, but larger hemorrhages at any site cause hypotonia and coma and signs of acute blood loss. Tears of the dural folds cause subdural hemorrhage only on the rare occasions when they extend into a sinus. However, they frequently coexist, as the same distorting force will also rupture bridging veins. Major SDH is not always due to trauma and can be seen in association with coagulation disorders such as alloimmune thrombocytopenia (see Chapter 8).

Tears of the Dural Folds

Excessive distortion of the head may also result in tearing of the tentorium, usually at its junction with the falx (Fig. 13.24), or more rarely of the falx itself. The dural folds support the brain, and

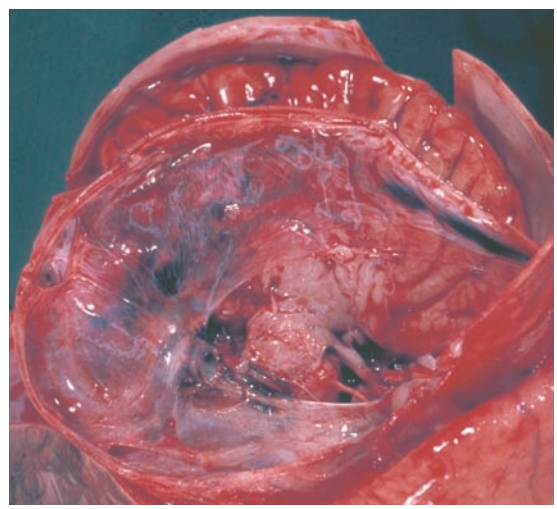

Figure 13.24. Tear at the angle of the right leaf of the tentorium and the falx. Instrumental delivery.

following a major tear this support may be reduced, permitting further distortion of the brain.

Dural tears are often accompanied by an SDH, which may be minor. However, the presence of a dural tear is a significant finding even when it involves only splaying of fibers within the fold. It is an indicator of prior head distortion due to the application of abnormal or unequal forces.

Hemorrhage into the falx and tentorium is a common postmortem finding; it is usually ischemic in origin and rarely extends to the surface.

Skull Fractures

The commonest skull fracture is an undisplaced linear break extending radially from the middle of the superior border of the parietal bone (Fig. 13.25). It usually complicates forceps delivery, especially where there has been fetopelvic disproportion. It is generally associated with other forms of traumatic or asphyxial birth injury. It occasionally complicates spontaneous delivery (Heise et al. 1996).

Depressed fracture (ping-pong fractures) can occur spontaneously but are more commonly found after instrumental delivery, particularly when forceps are used (Dupuis et al. 2005). Instrument-associated fractures are more frequently accompanied by intracranial pathology.

Comminuted fractures of skull vault bones are unusual, but are described following vacuum extraction (Hickey and McKenna 1966) and in-labor cesarean section when the head is impacted (Fig. 13.26).

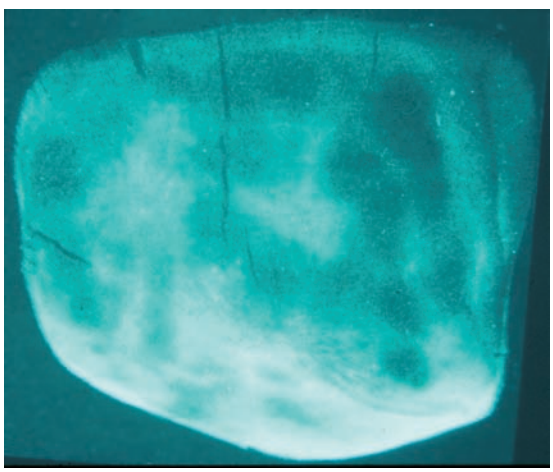

Figure 13.25. Radiograph of an excised parietal bone. Linear fractures run into the bone from its medial and posterior borders. Instrumental delivery.

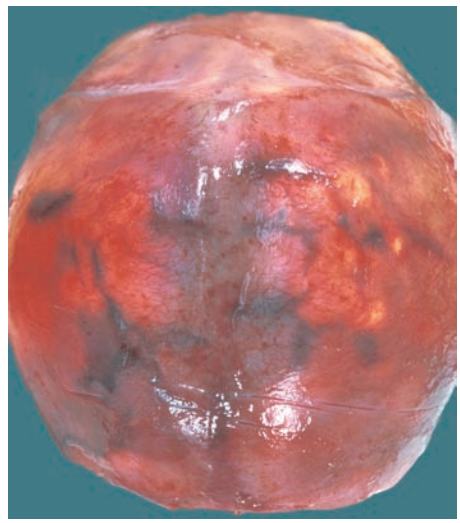

Figure 13.26. Irregular fractures of both parietal bones. Emergency cesarean section for failure to progress. The head was impacted.

Occipital Osteodiastasis

Excessive occipital pressure, usually during vaginal breech delivery but occasionally during forceps delivery, can cause separation of the cartilaginous joints between the squamous and lateral parts of the occipital bone. The result is inward and upward displacement of the inferior margin of the squamous part of the bone in respect of its lateral portions. Its effect is determined by the degree of displacement. The size of the posterior fossa may be reduced, sometimes with narrowing of the foramen magnum. Occipital venous sinuses may be torn and there may be laceration of the cerebellum (Wigglesworth and Husemeyer 1977). Major displacement is accompanied by other traumatic injuries such as dural tears. The diagnosis is often missed during life despite flattening of the occiput. The displacement may even go unrecognized during postmortem examination unless carefully sought (see Chapter 2) (Fig. 13.27).

Brain Emboli

Pulmonary embolization of brain tissue, usually cerebellum, is an uncommon complication of

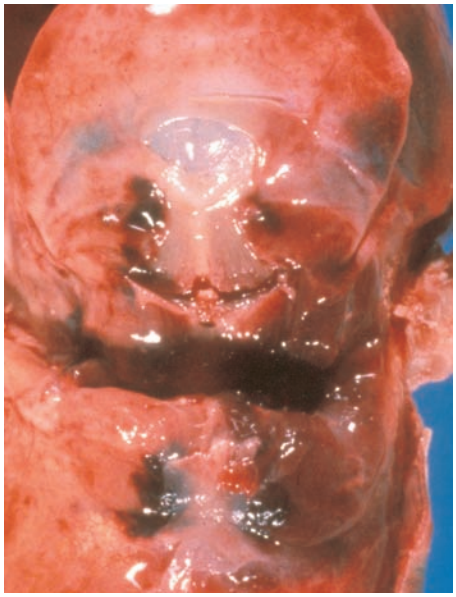

Figure 13.27. Occipital osteodiastesis. Inward displacement of the squamous part of the occipital bone is present. There is extensive local bruising. (Courtesy Dr. E.S. Gray, Aberdeen.)

cranial birth trauma, with less than 30 reported cases. It usually complicates breech or instrumental delivery. Damage to the brain or dural folds is not described in every case (Hauck et al. 1990; Baergen et al. 1997). Emboli are most commonly found in the lung (Fig. 13.28). Paradoxical embolization to coronary arteries is described and may precipitate rapid demise (Chan et al. 2003). Embolization of other vessels is sometimes seen. Disseminated intravascular coagulation provoked by the thromboplastin-rich emboli may complicate the picture in those babies surviving longer than 12 hours (Drut 1997). In two reports, embolization of cerebellar tissue to the placenta is recorded (Baergen et al. 1997; Silver and Newman 1998). In the latter, the infant survived with no neurological sequelae, raising the possibility that minor cerebellar embolism might go unnoticed, being identified only by consistent, detailed placental examination.

Older case reports document difficult delivery with considerable application of force (Baergen et al. 1997), but in Baergen et al.'s own case and in other reports excessive force was not a feature of delivery (Pillay 1980; Silver and Newman 1998; Chan et al. 2003). Tears of dural sinuses and major subdural hemorrhage are mentioned infrequently. Tryfus (1963) noted the superficial nature of the embolized fragments and thought it possible that ischemic cerebellar necrosis with vascular damage combined with raised intracranial pressure is sufficient to result in embolism and that major trauma is not necessary.

Spinal Cord Injuries

Injury to the spinal cord can result from hyperextension of the neck during vaginal breech delivery or ischemic injury or direct trauma during rotational forceps delivery (Fig. 13.29), although the etiology is often unclear (Ruggieri et al. 1999).

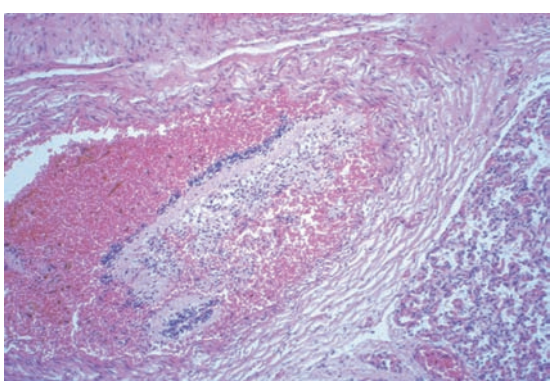

Figure 13.28. Lung. Fragments of cerebellar cortex are present in a large pulmonary artery. Instrumental delivery for failure to progress. Coronary artery embolism was also present.

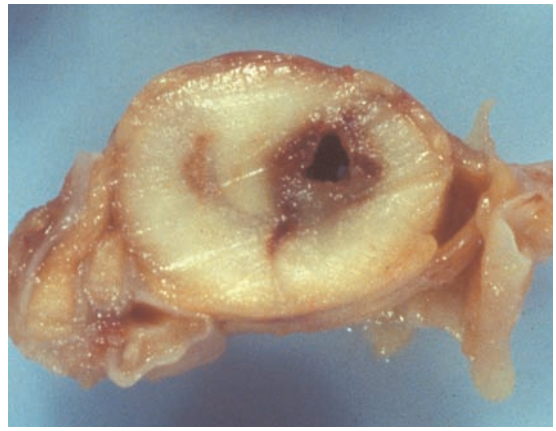

Figure 13.29. Spinal cord infarction. Emergency cesarean section for fetal distress.

Ebinger et al. (2003) describe cases following atraumatic delivery and suggest an unspecified pre- or perinatal ischemic etiology. With the increasing use of elective cesarean section for breech presentation, spinal cord trauma in this group is now uncommon. However, the possibility should be borne in mind when death follows difficult breech extraction. The commonest lesion after breech delivery is damage to the cord itself with hemorrhage and stretching of nerve roots in the middle or lower part of the cervical cord and occasional dural injury. Epidural hemorrhage is usually present. With cephalic presentation, damage is typically present in the upper cervical cord (MacKinnon et al. 1993; Mills et al. 2001), sometimes involving the medulla. When death follows delivery by rotational forceps, or the history is suggestive of spinal cord damage, careful examination of the medulla and cervical spinal cord, in situ prior to removal of the brain or the use of Yates's method (see Chapter 2), is advisable. Routine staining of the cord using immunohistochemistry for β-amyloid precursor protein to demonstrate axonal swelling is recommended.

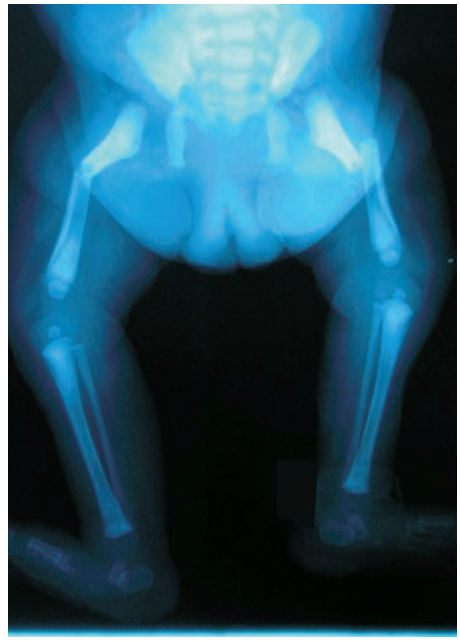

Figure 13.30. Bilateral femoral fractures following vaginal breech delivery. There is apparent shortening and lateral rotation of the lower limbs.

Visceral Injuries

A variety of other injuries may occur during delivery, the most common being clavicular fracture and damage to the brachial plexus and facial nerve. They are most commonly found in large babies in whom there is a history of obstetric manipulation and difficult delivery due to conditions such as shoulder dystocia (Oral et al. 2001; Raio et al. 2003). The injuries themselves are generally not fatal and are found postmortem in association with other, more serious injuries. Fractures of other bones occur in particular circumstances, for example, the humerus as an infrequent complication of shoulder dystocia, and the femur in extended breech or neurological abnormality (Fig. 13.30). In the immature fetus the blood loss following fracture of the femur or hemorrhage from visceral injury may be considerable and may contribute to severe postpartum shock. The enlarged, congested liver, spleen, and other internal organs of an asphyxiated infant with poor muscle tone are at much greater risk of rupture during parturition than are those of the normal baby. In very preterm babies, hemoperitoneum secondary to a ruptured subcapsular hematoma of the liver is not uncommon. Other forms of visceral injury are rare, but avulsion of the renal pedicle during precipitate delivery has been reported.

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14
Fetal Hydrops

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Fetal hydrops is an abnormal, generalized increase in interstitial fluid (edema) occurring prenatally and may be observed at any gestation from first trimester to term. There is usually an increase in total body water, and severe degrees of edema are often accompanied by effusions within the body cavities. Indeed, hydrops is usually recognized ultrasonographically by an increase in subcutaneous fluid in combination with an effusion in at least two serous cavities. Hydrops should be distinguished from localized postnuchal fluid blebs (increased nuchal translucency seen by ultrasound), which draw attention to a wide variety of fetal abnormalities that may later be complicated by fetal hydrops (see Chapter 6).

The incidence of generalized edema of the fetus is difficult to quantify. Minor fetal hydrops is very common, particularly among preterm neonates, and is often unexplained (Cartlidge and Rutter 1986). Major or severe fetal hydrops, defined as generalized edema or 5-mm subcutaneous edema in a third-trimester fetus and accompanied by effusions in at least one body cavity, was identified in one United Kingdom center in 1:1400 pregnancies when cases caused by rhesus incompatibility had been excluded (Iliff et al. 1983).

Clinical Presentation

The presentation of fetal hydrops is gestation related. In the first trimester of pregnancy, hydrops is observed incidentally in spontaneous abortions, during routine ultrasound scanning, and in scans prior to chorionic villus sampling. Until recently, fetal hydrops in the second trimester often presented with signs and symptoms related to intrauterine death of the fetus. It is now usually detected during ultrasound examination for estimation of fetal size and gestation, or prior to amniocentesis (Boyd and Keeling 1992; Jauniaux 1997). The majority of cases that present in the third trimester are discovered during the investigation of women who are "large for dates." Many of these women have clinically apparent polyhydramnios or rapid-onset preeclampsia (Keeling et al. 1983). In the third trimester, fetal hydrops often gives rise to premature onset of labor and third-stage complications, particularly postpartum hemorrhage and manual removal of the edematous placenta (Hutchison et al. 1982; Gough et al. 1986).

In rare cases, severe fetal hydrops may be accompanied by excessive maternal fluid retention (mirror syndrome; also known as Ballantyne syndrome, pseudotoxemia, and maternal hydrops syndrome). This is distinguished from preeclampsia, as there is usually maternal anemia due to hemodilution rather than hemoconcentration and earlier onset (~27 weeks). It is not usually associated with maternal proteinuria. Mirror syndrome arises in conjunction with fetal hydrops from various causes and can be fatal to both mother and fetus unless the underlying fetal condition is successfully treated or the pregnancy promptly terminated (Midgley and Harding 2000; Vidaeff et al. 2002). It is suggested that placental ischemia may account for the maternal manifestations, possibly by acting through factors produced by hyperproliferative trophoblast, such as human

chorionic gonadotrophin (hCG) (Gherman et al. 1998). However, the syndrome can develop in the presence of a normal placenta (Vidaeff et al. 2002).

Causes and Associations of Fetal Hydrops

Worldwide, α-thalassemia is the commonest cause of fetal hydrops (Thumasathit et al. 1968) and is still a major problem in Southeast Asia (Yang et al. 1998; Suwanrath-Kengpol et al. 2005). Until recently, the problem of fetal hydrops in Europe, North America, and Australia was dominated by the effects of rhesus incompatibility (Andersen et al. 1983). Following the introduction of effective management and prevention of rhesus disease with anti-D therapy (Whitfield 1981), other causes, taken together, are now numerically more important in those regions (Keeling et al. 1983; Machin 1989) and very many associations of fetal hydrops have been reported. These are listed in Tables 14.1, 14.2, and 14.3, but the majority of cases are associated with one or more of the following findings:

- Fetal anemia
- Chromosomal or other genetic abnormality
- Congenital anomaly (particularly cardiovascular)
- Infection
- Twin-to-twin transfusion syndrome
- Tumor

To underline the importance of particular associations, Keeling (2001) surveyed 30 reported series of 10 or more cases totalling 1645. To this survey is added the experience of Lallemand et al. (1999), Ismail et al. (2001), Sohan et al. (2001), Rodriguez et al. (2005) and Suwanrath-Kengpol et al. (2005). A summary of these series is presented in Table 14.4. These additional series illustrate the continuing geographic differences in associated pathology. They highlight the increase in underlying aneuploidy and a decrease in unexplained cases resulting from better and more consistent investigation of the hydropic fetus. Nineteen percent have chromosomal abnormalities, 17.1% have cardiovascular abnormalities, and 13.5% have anomalies in other systems.

Machin (1989) approached the problem in a different way. He surveyed nonselected series (804 cases) and individual case reports (610 cases) published between 1981 and 1988. His findings were similar, but he found more cardiovascular anomalies (26%); 12% had chromosome anomalies or suggestive dysmorphology and 12% had extracardiac anomalies.

Chromosomal Abnormality

During the last few decades, many more hydropic fetuses have been identified antemortem, improving the likelihood of determining an association or underlying cause. Furthermore, changes in the associations of fetal hydrops have been observed as a result of the increasing proportion of cases identified in the second trimester (Hansmann et al. 1989; Boyd and Keeling 1992). Until relatively recently, it is probable that many second trimester fetuses that were delivered some time after death were not submitted for pathological examination. Currently, there is a higher proportion of chromosomally abnormal fetuses (Jauniaux 1997) and of those with dysmorphic features suggestive of chromosome anomaly but unconfirmed by karyotyping (Boyd and Keeling 1992; Lallemand et al. 1999).

Fetal Malformation

A spectrum of fetal malformations has been described in association with fetal hydrops (Keeling et al. 1983; Machin 1989; Boyd and Keeling 1992). Many associated anomalies are likely to precipitate intrauterine heart failure in various ways, such as shunting through vascular malformations and cardiac abnormalities, which are likely to be accompanied by arrhythmias. In addition, fetal cardiac arrhythmias have been documented in both anatomically normal and malformed hearts (Kleinman et al. 1982; Naheed et al. 1996) in association with hydrops.

Many malformations that have been described in association with fetal hydrops have concomitant pulmonary hypoplasia affecting one or both lungs as an additional finding. Larroche (1982) posed the fundamental question: “Should the presence of the commonly associated finding (of

Table 14.1. Cardiothoracic and vascular disorders associated with fetal hydrops

Structural cardiac anomalies
Complex anomaly with ambiguous cardiac situs (Kleinman et al. 1982; Keeling et al. 1983)
Tetralogy of Fallot (Kleinman et al. 1982)
Hypoplastic left heart syndrome (Hutchison et al. 1982; Holzgreve et al. 1984)
Transposition of great arteries (Keeling et al. 1983; Holzgreve et al. 1984)
Truncus arteriosus (Im et al. 1984; Holzgreve et al. 1984)
Double outlet right ventricle (Schmid et al. 1988; Villaespesa et al. 1990)
Single ventricle (Holzgreve et al. 1984)
Pulmonary valve atresia (Ito et al. 1961; Allan et al. 1986)
Absent pulmonary valve + EFE (Moller et al. 1966; Kleinman et al. 1982)
Aortic valve stenosis/atresia (Moller et al. 1966; Moerman et al. 1982; Allan et al. 1986; Bitar et al. 1997)
Mitral atresia + EFE (Wilkin and Parmentier 1961)
Tricuspid valve atresia (Keeling et al. 1983; Holzgreve et al. 1984)
Tricuspid valve atresia and ectopia cordis (Allan et al. 1986)
Ebstein's anomaly (Moller et al. 1966; Allan et al. 1986)
Atrial septal defect (Holzgreve et al. 1984)
Atrioventricular canal defect (Kleinman et al. 1982; Allan et al. 1986)
Large ventricular septal defect (Etches and Lemons 1979; Holzgreve et al. 1984)
Arcadia (twin pregnancy) (Kleinman et al. 1982; Boyd and Keeling 1992)

Other cardiac pathology
Myocarditis (Keeling et al. 1983; Mostoufi-Zadeh et al. 1985)
Myocardial infarction (coronary artery embolus) (Kleinman et al. 1982)
Cardiomyopathy (Holzgreve et al. 1984; Allan et al. 1986)
Premature closure of foramen ovale (Naeye and Blanc 1964; Personen et al. 1983; Hagen 2005)
Premature closure of ductus arteriosus (Becker et al. 1977; Kohler 1978; Downing and Thibeault 1994)
EFE ± hepatitis (Spahr et al. 1980; Keeling et al. 1983)
Right atrial aneurysm (Barberato et al. 2005)
Cardiac diverticulum (Prefumo et al. 2005)

Cardiac tumors
Rhabdomyoma and tuberous sclerosis (Ostor and Fortune 1978; Kleinman et al. 1982; Keeling et al. 1983)
Cardiac teratoma (Mahony et al. 1984; Bruch et al. 1997)
Atrial angioma (Platt et al. 1981)

Cardiac arrhythmias
Supraventricular tachycardia (Radford et al. 1976; Kleinman et al. 1982)
Paroxysmal atrial tachycardia (Radford et al. 1976; Im et al. 1984)
Junctional ectopic tachycardia (Benito Bartolome and Jimenez Casso 2000)
Complete heart block (Etches and Lemons 1979; Holzgreve et al. 1984; Anandakumar et al. 1996a; Cooke et al. 1980)
Complete heart block and maternal connective tissue disease (McCue et al. 1977; Hardy et al. 1979)
Bradycardia (Im et al. 1984)
Bundle branch block (Cowan et al. 1975)
Arrhythmia and conduction system anomaly (Esscher and Scott 1979; Ho et al. 1985)
Tachyarrhythmia—virus induced (Ranucci-Weiss et al. 1998)
Wolff-Parkinson-White syndrome (Lauener et al. 1985)
Long QT syndrome (Miller et al. 2004)

Intrathoracic abnormalities
Pulmonary agenesis (Engellenner et al. 1989)
Tracheobronchomalacia (Phillips et al. 1985)
Adenomatoid malformation (Kleinman et al. 1982; Keeling et al. 1983)
Pulmonary sequestration (Etches and Lemons 1979; Kleinman et al. 1982)
Diaphragmatic hernia (Kleinman et al. 1982; Keeling et al. 1983)
Congenital high airway obstructive syndrome (CHAOS):
  Laryngeal or tracheal atresia (Keeling et al. 1983; Mostoufi-Zadeh et al. 1985)
  Mainstem bronchial atresia (Keswani et al. 2005)
Mesenchymal hamartoma (Khong and Keeling 1990)
Familial pulmonary lymphatic hypoplasia (Thibeault et al. 2002)

Vascular abnormalities
Hemangioma
  Fetus (Larroche 1977; Keeling et al. 1983)
  Fetus multiple (Shturman-Ellstein et al. 1978)
  Placenta (Jones et al. 1973; Keeling et al. 1983)
Atrial angioma (Platt et al. 1981)
Meningeal angiomatosis (Boyd and Keeling 1992)
Calcific arteriopathy (Ivemark et al. 1962; Keeling et al. 1983)
Descending thoracic aortic aneurysm (Nissen et al. 2000)
Abdominal aortic coarctation (Zeltser et al. 2003)
Pulmonary lymphangiectasia (Hutchison et al. 1982)
Diffuse lymphangiectasia (Laurence 1955; Windebank et al. 1987)
Cystic hygroma (Etches and Lemons 1979; Keeling et al. 1983)
Acardiac monozygous twin pregnancy (Kleinman et al. 1982; Boyd and Keeling 1992)
Venacaval thrombus (Rudolph and Levin 1977)
Superior caval vein obstruction (Adiotomre et al. 1994)
Agenesis of ductus venosus (Siven et al. 1995; Durakovic et al. 2005)
Abnormal course of umbilical vein (Hofstaetter et al. 2000)
Agenesis of portal or hepatic veins (Hofstaetter et al. 2000)
Abnormal course of inferior vena cava (Hofstaetter et al. 2000)
Coronary artery embolus (Kleinman et al. 1982)
Fetal entanglement with umbilical cord (Keeling et al. 1983)
Umbilical cord torsion (Collins 1995)

EFE, endocardial fibroelastosis.

Table 14.2. Other fetal disorders associated with fetal hydrops

Gastrointestinal
Esophageal atresia (Larroche 1982)
Jejunal atresia (Spahr et al. 1980)
Small intestinal volvulus (Seward and Zusman 1978)
Meconium ileus ± peritonitis (Etches and Lemons 1979; Hutchison et al. 1982)
Intestinal infarction (Holzgreve et al. 1984)
Intestinal obstruction (Mahony et al. 1984)
Hepatitis (Hutchison et al. 1982; Keeling et al. 1983)
Hepatic necrosis (Hutchison et al. 1982)
Cirrhosis (Spahr et al. 1980; Keeling et al. 1983)

Urogenital abnormalities
Cystic renal dysplasia (Larroche 1982)
Renal hypoplasia (Etches and Lemons 1979; Keeling et al. 1983)
Urethral obstruction
- Valves (Holzgreve et al. 1984)
- Atresia (Keeling et al. 1983)

Congenital nephrotic syndrome (Worthen et al. 1959; Mostoufi-Zadeh et al. 1985)
Meckel syndrome (Boyd and Keeling 1992)
Fraser syndrome (Potter 1943; Keeling et al. 1983)
Vaginal atresia, hydrometrocolopos syndrome (Hutchison et al. 1982; Moerman et al. 1982)
Autosomal recessive kidney disorder (Van Maldergem et al. 1992)
Mesoblastic nephroma (Larroche 1977; Gray 1989)

Musculoskeletal
Achondrogenesis I (Mostoufi-Zadeh et al. 1985)
Achondrogenesis II (Golbus et al. 1977; Moerman et al. 1982)
Osteogenesis imperfecta (Hutchison et al. 1982; Mostoufi-Zadeh et al. 1985)
Thanatophoric dysplasia (Fleischer et al. 1981)
Asphyxiating thoracic dystrophy (Jeune) syndrome (Hutchison et al. 1982)
Short rib-polydactyly, type I (Saldino-Noonan) syndrome (Richardson et al. 1977)
Greenberg dysplasia (Chitayat et al. 1993)
Ellis–Van Creveld syndrome (Mahony et al. 1984)
Congenital cortical hyperostosis (Caffey disease) (Lecollier et al. 1992)
Multiple pterygium syndrome (Holzgreve et al. 1984)
Popliteal pterygium syndrome (Mahony et al. 1984)
Fetal akinesia deformation sequence (Pena-Shokeir, type I phenotype) (Holzgreve et al. 1984)
Neu-Laxova syndrome (Holzgreve et al. 1984)
Myotonic dystrophy (Mahony et al. 1984)
Myopathy with muscle spindle excess (Stassou et al. 2005)
Amniotic bands (Holzgreve et al. 1984)
Simpson-Golabi-Behmel syndrome (Terespolsky et al. 1995)
Oral-facial-digital syndrome (Van Maldergem et al. 1992)
Noonan syndrome (Katz et al. 1993)

Tumors
Neuroblastoma (Anders et al. 1973; Moerman et al. 1982)
Cardiac rhabdomyoma (Ostor and Fortune 1978; Keeling et al. 1983)
Hepatoblastoma (Kazzi et al. 1989)
Hepatic mesenchymal hamartoma (Kamita et al. 2003)
Primitive neuroectodermal tumor (Boyd and Keeling 1992)
Teratoma
- Sacrococcygeal (Kohler 1976; Hutchison et al. 1982)
- Mediastinal (Beischer et al. 1971; Fleischer et al. 1981)
- Intrapericardial (Holzgreve et al. 1984; Mahony et al. 1984; Bruch et al. 1997)

Congenital leukemia, Down syndrome (Donnenfeld et al. 1994; Zipursky et al. 1996)
Mesoblastic nephroma (Larroche 1997; Gray 1989)
Nephroblastoma (Vadeyar et al. 2000)
Rhabdoid tumor of the kidney (Castellino et al. 2001; Fuchs et al. 2003)
Retroperitoneal kaposiform hemangioendothelioma (Martinez et al. 2004)
Cervical fibrosarcoma (Lam et al. 2004)
Glioblastoma multiforme (Sabet 1982)

Other
Hydrocephaly, arthrogryposis (Holzgreve et al. 1984)
Holoprosencephaly (Mostoufi-Zadeh et al. 1985)
Central nervous system destruction, hypomobility (Robin et al. 1994)
Hyperthyroidism (Treadwell et al. 1996)
Incontinentia pigmenti (Dufke et al. 2001)

fetal hydrops) of pleural effusion, be considered a cause or a consequence of pulmonary hypoplasia?" but did not answer it. She drew attention to the more common association of pulmonary hypoplasia and oligohydramnios and to the role of pulmonary secretions in the production and composition of amniotic fluid. The suggested relationship of lung hypoplasia and hydrops, based on observation of many of the associated anomalies, is that there is a common effect of reduced thoracic volume with hydrops subsequently occurring because high intrathoracic pressure impedes venous return from the placenta. The role of lung hypoplasia is therefore probably that of an innocent bystander.

Intrauterine Infection

Fetal hydrops is an uncommon but serious manifestation of intrauterine infection in the U.K., although the importance of parvovirus B19 as a cause of fetal hydrops during epidemics is widely recognized (Morey et al. 1992; Rogers et al. 1993; Wright et al. 1996; Walters et al. 1997), and persis-

Table 14.3. Other associations of fetal hydrops

Genetic metabolic disease
Infantile Gaucher (Im et al. 1984; Mostoufi-Zadeh et al. 1985)
Mucopolysaccharidosis (Mahony et al. 1984; Nelson et al. 1993; Molyneux et al. 1997)
Sialidosis (Johnson et al. 1980; Beck et al. 1984)
Galactosialidosis (Michelakakis et al. 2004)
Niemann-Pick (Rossier et al. 1958)
GM_1, gangliosidosis (Etches and Lemons 1979)
Hypoalbuminemia (Turski et al. 1978)
Long-chain acyl-coenzyme A dehydrogenase deficiency (LCHAD) (Tercanli et al. 2000a)
Fatal perinatal neuromuscular glycogenosis (type IVA) (Giuffra et al. 2004)
Carnitine deficiency (Steenhout et al. 1990)
Neonatal hemochromatosis (Wisser et al. 1993)
Congenital erythropoietic porphyria (Gunther disease) (Verstraeten et al. 1993)
Congenital disorder of glycosylation type Ia (Noelle et al. 2005)

Chromosome anomalies
45XO (Kleinman et al. 1982; Mahony et al. 1984)
Trisomy 21 (Moerman et al. 1982; Holzgreve et al. 1984)
Trisomy 18 (Macafee et al. 1970; Keeling et al. 1983)
Trisomy 13 (Sahn et al. 1982; Mahony et al. 1984)
Trisomy 15 (Kuller and Laifer 1991)
Triploidy (Hutchison et al. 1982)
Unbalanced 3;7 translocation (Willekes et al. 1994)
1p36 deletion (Faivre et al. 1999)
Distal 5q duplication (Witters et al. 1998)
6p deletion (Suwanrath-Kengpol et al. 2004)
Trisomy 10 mosaicism (Knoblauch et al. 1999)
Interstitial deletion 10q22.3-q24.1 (Jain and Jones 2001)
Pallister-Killian syndrome (tetrasomy 12p) (Langford et al. 2000)
Unbalanced 8;11 translocation (Fert-Ferrier et al. 2000)
Partial trisomy 19q (Tercanli et al. 2000b)
22p11 microdeletion (Machlitt et al 2002)
90XX (Syhez et al. 1999)

Hematological disorders
Inherited
- Rhesus incompatibility (Whitfield 1981)
- ABO incompatibility (McCue et al. 1977; Huchet and Soulié 1979)
- Kell incompatibility (Miser et al. 1975; Scanton and Muirhead 1976)
- α-Thalassemia (Thumasathit et al. 1968; Holzgreve et al. 1984)
- Hemoglobin Bart's (Weatherall et al. 1970; Boer and Anido 1979)
- Hemoglobin Bart's + H (Lie and Lie 1961)
- Hemophilia A (Mahony et al. 1984)
- Pyruvate kinase deficiency (Hennekam et al. 1990; Ferreira et al. 2000)
- Glucose-6-phosphate dehydrogenase deficiency (Perkins 1971; Mentzer and Collier 1975)
- Diamond-Blackfan syndrome (Scimeca et al. 1988; McLennan et al. 1996)
- Dyserythropoietic anemia (Carter et al. 1989; Cantu-Rajnoldi et al. 1997)

Congenital xerocytosis (Vincente-Gutierrez et al. 2005)
Transient myeloproliferative disorder in Trisomy 21 (Smrcek et al. 2001)

Acquired
Chronic fetomaternal hemorrhage (Debelle et al. 1977; Mostoufi-Zadeh et al. 1985)
Fetomaternal hemorrhage (choriocarcinoma) (Blackburn 1976; Nieuwenhuijzen Kruseman et al. 1977)
Fetofetal hemorrhage (twin-transfusion syndrome) (Keeling et al. 1983; Mahony et al. 1984)
Hemorrhage into fetal organs
- Intracranial with ischemia (Bose 1978; Etches and Lemons 1979; Coulson et al. 1994)
- Intracranial with tumour (Boyd and Keeling 1992)
- Intraabdominal (Seward and Zusman 1978)

Infection
Parvovirus B19 (Burton 1986; Porter et al. 1988; Morey et al. 1992)
CMV (Hutchison et al. 1982; Holzgreve et al. 1984)
Human herpes virus type 1 (Im et al. 1984)
Rubella virus (Spahr et al. 1980)
Toxoplasmosis (Bain et al. 1956; Spahr et al. 1980)
Syphilis (Bulova et al. 1972; Bryan and Nicholson 1981)
Coxsackie virus (Bates 1970)
Myocarditis NOS (Keeling et al. 1983; Mostoufi-Zadeh et al. 1985)
Adenovirus (Towbin et al. 1994; Ranucci-Weiss et al. 1998)
Candida (Silver et al. 1994)

Maternal disorders
Rheumatological disorders (Altenberger et al. 1977; Hardy et al. 1979)
Antiphospholipid antibody syndrome (Hage et al. 1994)
Diabetes mellitus (Graves and Baskett 1984)
Maternal anemia with two hydropic pregnancies (Macafee et al. 1970; Larroche 1982)
Choriocarcinoma (Zarafu et al. 1978)

CMV, cytomegalovirus; NOS not otherwise specified.

tent infection may result in anemia due to red-cell aplasia, which may be fatal (Brown et al. 1994). Cytomegalovirus and fetal bacterial infection continue to cause a small number of fetal deaths due to hydrops (Lallemand et al. 1999; Rodriguez et al. 2005). While syphilis is still a numerically important cause of hydrops in parts of Southeast Asia (Bryan and Nicholson 1981) and South America (Garcia et al. 1996), it is still seen sporadically elsewhere (El Tabbakh 1994). An occasional association with *Chlamydia* infection is recognized in Western populations (Lallemand 1999).

Table 14.4. Associations of nonimmune fetal hydrops: a summary of 34 published series of 10 or more cases

	Keeling 2001 (summary of 30 published series)	Lallemand et al. 1999 (France) (fetal and perinatal autopsies)	Sohan et al. 2001 (UK)	Ismail et al. 2001 (UK)	Rodrigez et al. 2005 (Miami, FL) (liveborns)	Suwanrath-Kengpol et al. 2005 (south Thailand)	Total No.	%
Cardiovascular abnormalities[a]	287	13	11	5	16	7	339	17.1
Noncardiovascular anomalies[b]	219	11	12	14	7	5	268	13.5
Chromosomal abnormalities	297	19 confirmed/ 31 suspected	28	14		7	377	19.0
Twin-to-twin transfusion (monochorionic twins)	90	8	5	2	1	3	109	5.5
Infection	143	15	13	8	6	9	194	9.8
Fetal anemia	115		5	1		20	141	7.1
Tumors	24		2				26	1.3
Hepatic pathology	16						16	0.8
Genetic metabolic disease	15						15	0.8
Meconium peritonitis	18						18	0.9
Other[c]		7	2	6		4	19	1.0
Unexplained	421	9	9	8	2	9	458	23.1
Total	1645	94	87	55	32	71	1984	100

[a]Includes fetal arrhythmia.
[b]Includes chylothorax.
[c]Includes uteroplacental insufficiency and maternal diabetes.

Distribution and Control of Fetal Fluid

Water is the major constituent of the human body. Its contribution to body mass changes with maturity, comprising 88% of fetal mass at 18 to 20 weeks' gestation and 73% at term (Friis-Hansen 1971). Water may be intracellular or extracellular, the latter comprising both the intravascular compartment and the interstitial space. The volume of the intracellular compartment depends on the relationship between osmotic forces both within and outside of the cell, and on a functioning sodium pump within cell membranes.

The distribution of extracellular water between interstitial and intravascular compartments is dependent on a variety of mechanisms, and the controlling factors are clearly more complex than that postulated by Starling. Mayerson et al. (1960) suggested that bulk flow of water accompanies protein molecules across capillaries in the lung and liver. At these sites, the capillary pores are large enough to permit the passage of protein molecules. In other sites, water escapes from the intravascular compartment by simple diffusion through intercellular clefts in the capillary wall.

The stability of the relationship between interstitial and intravascular fluid depends on the structure and components of the interstitial space. This comprises a gel of mucopolysaccharide molecules (largely hyaluronic acid) lying in the spaces of a collagen network. The gel contains protein and salts, particularly sodium ions, and is only partially saturated with water. Normally, no free fluid is present within the interstitial space. Water is attracted into the interstitium by the partially saturated gel, and removed by the small, negative hydrostatic force exerted by the

pumping action of skeletal muscle acting on lymphatics.

Small changes in the water content of the matrix produce large changes in interstitial fluid pressure until saturation of the gel occurs. At this point, a large increase in water content has little effect on interstitial fluid pressure (Guyton and Hall 1996), but should more water enter the interstitial space, pooling of free fluid and, consequently, edema, occurs. This fluid can move through between the tissues under the influence of gravity.

Amniotic Fluid Dynamics

Amniotic fluid volume increases as pregnancy progresses, although not at a constant rate. Its chemical composition alters as the fetus matures. The relationship among fetal size, weight, and amniotic fluid volume also changes with increasing fetal maturity.

During embryogenesis, amniotic fluid volume is greatly in excess of fetal volume. A fluid-filled amniotic cavity is recognizable around the time of implantation, before the embryo is distinguishable (Boyd and Hamilton 1970). Amniotic fluid is not dependent on the embryo; fluid continues to accumulate both when it fails to develop and when it is resorbed and is thought to be secreted by the amnion.

In the early fetal period, amniotic fluid volume is closely related to fetal size (Abramovich 1968; Lind et al. 1972). It increases from 25 mL at 10 weeks' gestation to around 400 mL at 20 weeks; its composition closely resembles fetal plasma. There is rapid diffusion of water between the fetus and the amniotic fluid, estimated at 400 to 500 mL per hour. This is grossly in excess of the net increase in amniotic fluid volume (about 0.5 mL per hour, assuming a steady increase of about 100 mL per week). In the first half of pregnancy, fetal skin and umbilical cord amnion are freely permeable to water and solute (Page et al. 1978). In the rhesus monkey, amniotic fluid volume may change in order to maintain the tonicity of fetal extracellular fluid (Schruefer et al. 1972). Before midpregnancy, it is unlikely that fetal swallowing or micturition exerts any influence on either the composition or the volume of amniotic fluid.

In the second half of pregnancy, the relationship between fetal size and amniotic fluid volume is no longer linear. While there is around 800 mL of fluid at term, its rate of increase is outstripped by that of fetal size and weight. Keratinization of fetal skin begins at 19 to 20 weeks' gestation and is well developed by 25 weeks. Free diffusion of water and solutes through the skin is then no longer possible. The amniotic fluid loses its function both as a physiological buffer and as an extension of the fetal extracellular compartment. It becomes an externalized fluid affording physical protection. Its volume is increased continually by voiding of fetal urine; a similar volume is removed by fetal swallowing.

There is exchange of fluid across the respiratory tract during the third trimester. This has largely been ignored and its contribution to amniotic fluid volume has not been determined.

During the last trimester of pregnancy, fetal anomalies that interfere with swallowing are often accompanied by polyhydramnios. Anomalies that result in reduced voiding of urine are associated with oligohydramnios. It is known that water can cross both umbilical cord amnion and the chorionic plate of the placenta close to term (Abramovich and Page 1973). However, while there is no evidence to suggest that fluid exchange at either site modifies amniotic fluid volume in the presence of a healthy fetus, such routes might be important when this is not the case.

The volume of amniotic fluid falls rapidly after 40 weeks' gestation to around 400 mL at 42 weeks and then to 200 mL at 44 weeks (Queenan et al. 1972). The reason for this has not been determined, nor is there any clear relation to increased fetal mortality in postterm gestation.

Mechanisms of Fetal Hydrops

Consideration of the types of pathological abnormality in published series of fetal hydrops identifies the common features from which causal mechanisms may be inferred (Keeling et al. 1983). The three most important failures of fetal homeostasis that are associated with hydrops are *severe chronic anemia*, for example, rhesus incompatibility (Whitfield 1981) and α-thalassemia

(Thumasathit et al. 1968); *hypoproteinemia*, for example, congenital nephrotic syndrome (Worthen et al. 1959); and *intrauterine heart failure*, for example, high-output failure through arteriovenous anastomoses (Knoth et al. 1976) or cardiac arrhythmias (Fleischer et al. 1981). While any one of these three mechanisms may be the initiating factor, complications arise as fluid balance deteriorates. Eventually all three mechanisms may contribute to worsening of the hydropic state. Phibbs et al. (1974) found that the severity of the hydrops in severe rhesus disease correlated better with serum albumin level than with red cell volume, and Gordon et al. (1966) found that they could not reverse rhesus hydrops when adequate hemoglobin levels were achieved and maintained by intrauterine transfusion, suggesting that irreversible capillary damage had already occurred. Moise et al. (1992) compared hematocrit and colloid osmotic pressure in hydropic and nonhydropic anemic fetuses. In addition to lower levels of both in the hydropic fetuses, there was also a small increase in umbilical venous pressure. The authors concluded that minor abnormalities of Starling forces had a role in the development of hydrops in the anemic fetus. These observations are relevant to the poor response to treatment often observed in fetal hydrops.

In addition to the three mechanisms outlined above, there is little doubt that a variety of structural abnormalities that primarily interfere with the fetoplacental circulation, usually obstructing venous return from the placenta, are causally related to fetal hydrops. It seems that obstruction to venous return from the placenta initiates a series of events:

Increased venous pressure →
Placental edema →
Impaired gas exchange →
Fetal hypoxia →
Fetal capillary damage →
Loss of plasma proteins →
Fetal hydrops

Examples of this situation include premature closure of the ductus arteriosus (Kohler 1978), right-sided diaphragmatic hernia (Keeling et al. 1983), and abdominal tumors (Moerman et al. 1982). This mechanism has some experimental support. In one study, fetal tracheal occlusion was performed to stimulate lung growth in a fetus with diaphragmatic hernia. The consequent rapid lung expansion resulted in cardiac compression and hydrops (Graf et al. 1997).

Edema of the placenta interferes with maternofetal nutrient and gaseous transfer (Bryan 1977), and this may contribute to the worsening of the fetal condition in two ways: poor amino acid/peptide transport may contribute to decreased fetal plasma protein levels, and less efficient oxygenation may produce hypoxic damage to fetal capillaries and loss of plasma proteins into the interstitial space.

It is likely that the time of onset and rate of change of a particular initiating mechanism matters as much as its ultimate abnormal value. The rarity of hydrops in analbuminemia and erythrogenesis imperfecta, where levels of serum albumin and hemoglobin respectively are extremely low throughout pregnancy, lends support to this suggestion (Barnes et al. 1977).

Investigation of Fetal Hydrops

The underlying cause of hydrops is more likely to be discovered in those cases where it is observed while the fetus is still alive because a much wider range of investigations can be undertaken. The increasing use of ultrasound examination has resulted in a marked reduction in the number of cases designated "unexplained" (Fleischer et al. 1981; Gough et al. 1986; Boyd and Keeling 1992; Iskaros et al. 1997) when compared with earlier studies where ultrasound examination was not routine (Macafee et al. 1970; Hutchison et al. 1982). Serial ultrasound examination has documented the spontaneous resolution of even severe degrees of fetal hydrops (Platt et al. 1978; Iliff et al. 1983).

The aims of investigations following the discovery of fetal hydrops are to ascertain the underlying cause or association and to document the severity of fetal edema, the presence or absence of effusions in body cavities, the degree of polyhydramnios and the extent of placental involvement so that rational decisions can be made about patient management and estimates

of risk of recurrence given (Holzgreve et al. 1984).

Some urge immediate delivery when a diagnosis of fetal hydrops is made in the third trimester, arguing that pulmonary hypoplasia secondary to pleural effusions constitutes the major risk to the fetus (Andersen et al. 1983). Others advocate careful ultrasound examination to identify the fetus with major malformation (Fleischer et al. 1981; Kleinman et al. 1982). Mahoney et al. (1984) found that they were unable to predict outcome on the basis of ultrasonographic examination alone.

Immediate investigation with elective delivery, or medical intervention and careful, repeated observation was advocated by Gough et al. (1986) in view of the high incidence of major malformations that have a poor outcome even in the most favorable circumstances, the difficulties encountered during resuscitation of the hydropic infant, and the impact of cesarean section on the mother's reproductive career. Spontaneous resolution of fetal hydrops can occur with excellent outcomes for both mother and fetus (Platt et al. 1978; Keeling et al. 1983). Anandakumar et al. (1996b) advocated a selective approach to therapy aimed at fetal salvage based on detailed prenatal investigation. They identified an underlying cause for hydrops prenatally in 81% of their cases and 18 of 26 fetuses (69%) receiving in utero therapy survived.

Prenatal Therapy for Fetal Hydrops

A number of different types of prenatal intervention for the treatment or prevention of fetal hydrops have been tried, with some success to date. As the development of hydrops is often seen as a poor prognostic feature, particularly in conjunction with complex structural heart defects (Berg et al. 2005; Maeno et al. 2005), the need for rapid and specific assessment of the underlying problem is underlined.

Among the earliest types of prenatal therapy tested were antiarrhythmic drugs, such as digoxin or beta-blockers administered to the mother, in cases of fetal arrhythmia (Rees et al. 1980; Fleischer et al. 1981; Iliff et al. 1983). Digitalization was also useful in a case of severe aortic stenosis with endocardial fibroelastosis at 27 weeks and resulted in the resolution of hydrops and a live-birth delivery at term (Schmider et al. 2000). Digoxin has also been useful in the management of congenital cystic adenomatoid malformations (CCAMs) of the lung in conjunction with neonatal surgery (Entezami et al. 1998). In cases of fetal tachycardia (reentrant supraventricular, junctional ectopic, or ventricular) refractory to digoxin, the addition of maternal oral amiodarone can be effective in fetal hydrops or ventricular dysfunction (Strasburger et al. 2004). Antiarrhythmic drugs, including digoxin, have been given to fetuses directly to counteract the often poor or unpredictable placental drug transfer that may occur in the hydropic fetoplacental unit (Hansmann et al. 1989; Petrikovsky et al. 1996). Direct fetal therapy with amiodarone may benefit supraventricular tachycardia and atrial flutter in hydrops and its use reduces the number of repeat administrations required owing to its prolonged elimination half-life (Hansmann et al. 1991). Autoimmune-associated atrioventricular block has been managed by immunoadsorption of maternal anti-Ro/SSA antibodies (Hickstein et al. 2005).

Other drugs, particularly corticosteroids, may have utility depending on the etiology of hydrops. Hydrops due to CCAMs of the lung, including in Stocker type III, which is usually the most difficult type to manage, has been observed to resolve following administration of betamethasone (Tsao et al. 2003; Leung et al. 2005 and references therein). It has been speculated that the response to such therapy owes its effect to a degree of lesional maturation or involution (Tsao et al. 2003). Steroid medication (Scott et al. 1997) and aggressive amnio-reduction (Duncan et al. 1997) may have a role in the management of twin-to-twin transfusion with hydrops.

An emerging modality in fetal cardiac intervention is in utero cardiac catheterization combined with detailed fetal Doppler ultrasound assessment. Miniaturization of balloon catheters has the potential for salvage of conditions such as pulmonary stenosis and biventricular outflow tract obstruction and may prevent the development of hydrops in these conditions as well as contribute to the treatment of fetal aortic stenosis, which is thought to be a precursor lesion in

the hypoplastic left heart syndrome (Huhta et al. 2004).

Thoracoamniotic drainage of pleural effusions/chylothorax or large cysts (Brown et al. 1995; Ryo et al. 1997) to encourage lung growth is now relatively frequently undertaken, with survival (57%) in one study limited mainly by premature labor (Picone et al. 2004). Successful outcomes have also been achieved with a similar approach in pulmonary sequestration (Favre et al. 1994; Salomon et al. 2003). Pericardiocentesis and thoracoamniotic shunting to relieve tamponade has been performed with intrapericardial teratomata enabling tumor resection in the neonate (Sklansky et al. 1997; Grebille et al. 2003). Likewise, drainage of ascites or the urinary bladder in order to reduce intraabdominal pressure may be beneficial.

Open fetal pulmonary lobectomy for CCAM has been successful with a fetal survival rate of about 50% to 60% (Adzick et al. 2003). A relatively new surgical procedure known as EXIT (ex utero intrapartum treatment) is beginning to enable salvage of hydropic fetuses with congenital high-airway obstruction syndrome (CHAOS) (Crombleholme et al. 2000; Lim et al. 2003).

Hydrops is an indication in some centers for intervention in cases of sacrococcygeal teratoma owing to high fetal mortality rates. Percutaneous drainage of large cysts may alleviate dystocia at delivery and can decompress the renal system in cases where there is a cystic intrapelvic component. Prenatal open total or partial resection of the tumor has been performed in a small number of reported cases but may give way in the future to feeding vessel ablative therapy or fetoscopic resection to limit the risks to mother and fetus of mid-gestation hysterotomy (Hirose and Farmer, 2003).

Fetal blood transfusion and albumin infusion have been used in various circumstances such as parvovirus B19 infection (Soothill 1990; Fairley et al. 1995), ascitic/hydropic RhD alloimmunization (Craparo et al. 2005), and idiopathic hydrops (Shimokawa et al. 1988). Radiofrequency ablation of the blood flow to an acardiac twin was an effective therapy for the hydropic pump twin (Hirose et al. 2004), as was alcoholization of the feeding vessel of a placental chorangioma (Wanapirak et al. 2002). Sclerotherapy with OK-432 was of benefit in an enlarging cystic hygroma (Kubawara et al. 2004).

Table 14.5. Investigations for fetal hydrops

Prenatal investigations	Investigation of the mother by ultrasonography
Hemoglobin, Kleihauer	Severity of hydrops
ABO and rhesus group	Severity of polyhydramnios
Serum α-fetoprotein	Multiple pregnancy
Hemolysins, hemagglutinins	Fetal heart rate/rhythm
Minor blood group antigens	Fetal anomaly
Antinuclear factor	Heart
Autoantibodies	Other
Serological tests for syphilis	Placenta
Placental biopsy/amniocentesis	Thickness
Chromosomes	Other abnormality
Virus culture	

When the diagnosis of fetal hydrops is made, the investigations listed in Table 14.5 should be undertaken as a matter of urgency. All the investigations should be undertaken, even though results might have been normal earlier in pregnancy. The investigations identify the small group of cases where immediate delivery or active antenatal management may be lifesaving. These conditions include minor blood group abnormalities (anti-e, -b, or -c, or Kell), fetal cardiac arrhythmias, placental chorioangioma, twin-to-twin transfusion syndrome, fetomaternal hemorrhage, maternal anemia, and diabetes mellitus. Late-referral of alloimmunized hydropic fetuses results in reduced chances of survival (van Kamp et al. 2004).

Table 14.6. Investigation of the hydropic neonate

At birth	Necropsy
Full blood count	Photographic record
Blood group and antibodies	Weight and measurements
Clotting screen	Malformation
Liver function tests	Cardiac
Viral antibodies	Other
WR, karyotype	Lung weight
Echocardiogram	Histological examination
Radiological examination	Virus culture and antibodies
Cardiac catheterization	Blood group and antibodies
Effusions	Effusions
Culture	Culture
Biochemical analysis	Biochemical analysis

WR, Wasserman reaction.

Amniocentesis with controlled removal of fluid may contribute to maternal comfort, decrease the likelihood of spontaneous premature onset of labor, and yield samples for microbiological culture and biochemical examination, which may assist management.

When a hydropic fetus is born alive and the underlying cause of hydrops is not apparent, the investigations listed in Table 14.6 should be contemplated. Essential postmortem investigations are also listed in Table 14.6.

Pathological Findings

External

The affected fetus is pale and usually preterm. The amount and distribution of soft tissue edema is variable, the head and face are often markedly affected while the limbs are sometime spared. When edema is gross (Fig. 14.1; also see Fig. 14.3B), the deforming effect, particularly of the head, both obliterates dysmorphic features, such as the facies associated with some chromosome anomalies,

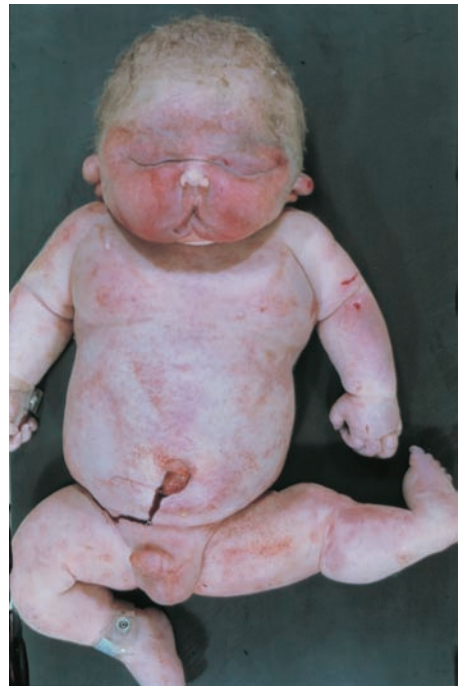
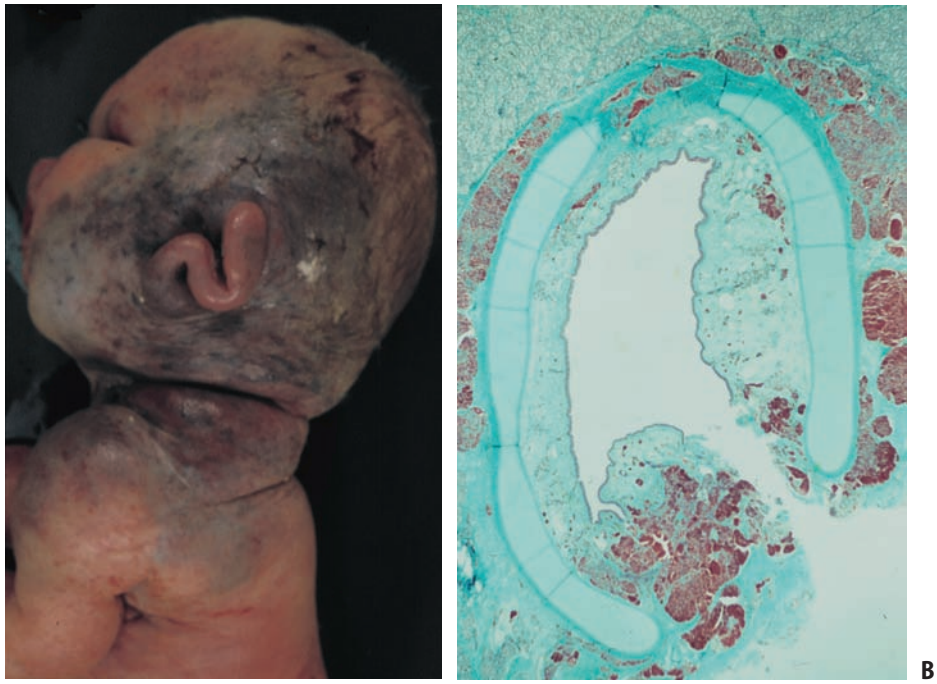


Figure 14.1. Gross hydrops involving head, trunk, and limbs. Severe edema introduces facial deformity.



A B

Figure 14.2. (A) Hydropic stillbirth with a large angioma involving the left side of the face, head, and shoulder. (B) The angioma involved the thyroid and soft tissue within and around the larynx.

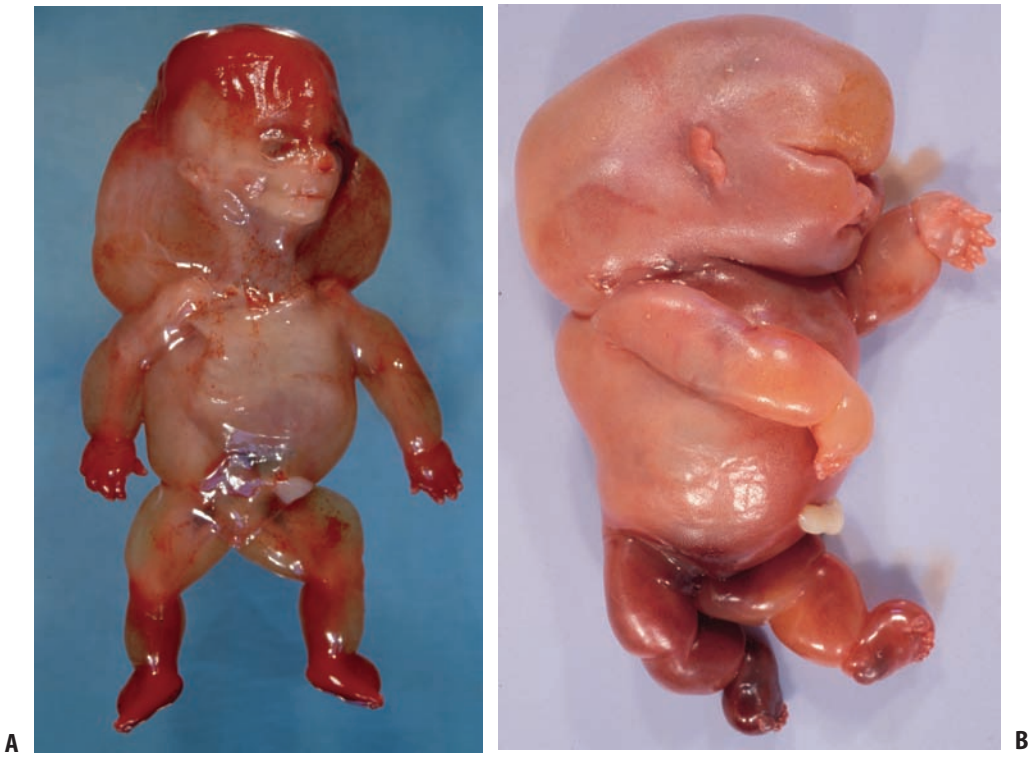


Figure 14.3 (A) Fetus with generalized hydrops and marked post-nuchal fluid accumulation; karyotype was 45XO. (B) Another 45XO fetus, later in gestation, with generalized edema extending onto the hands and feet. Note displacement of the ear anteriorly by the edema fluid.

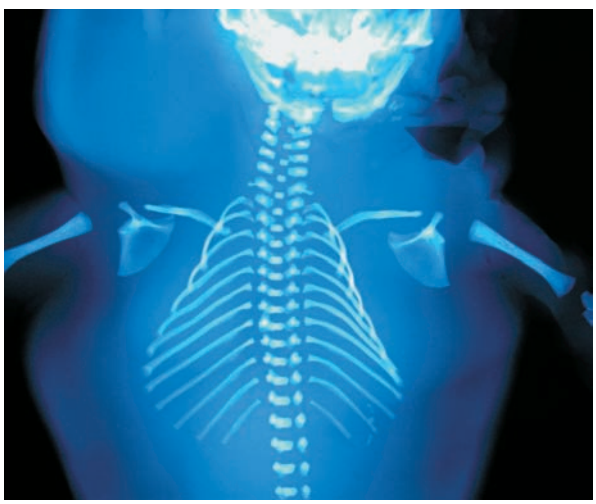


Figure 14.4. Postmortem radiograph of a fetus with 45XO karyotype showing marked soft tissue expansion due to edema. Note the bilateral cervical ribs.

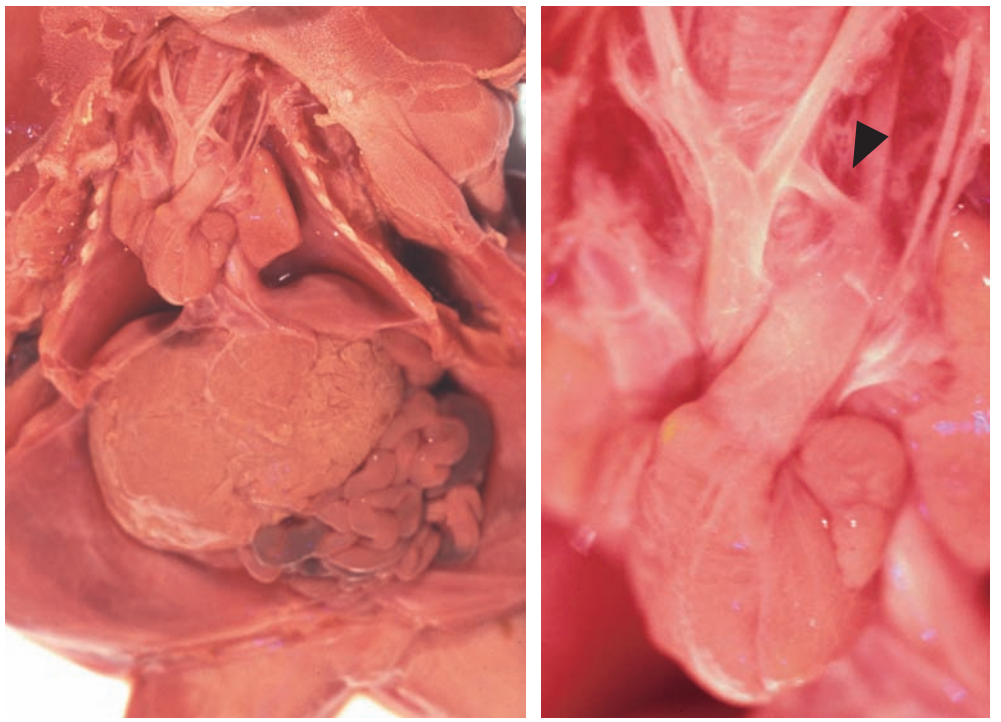


Figure 14.5. (A) Hydropic fetus with 45XO karyotype. The pleural and peritoneal cavities are enlarged, and the lungs and intestines are compressed owing to pleural effusions and ascites. (B) The pulmonary trunk is larger than the ascending aorta and the distal part of the aortic arch is very narrow (tubular hypoplasia) (arrowhead).

and produces changes, such as ear folding, which may be wrongly interpreted as dysmorphism. Gross external abnormalities, for example, skeletal dysplasia (see Fig. 29.21 and Fig. 29.22 in Chapter 29) or extensive angiomata (Fig. 14.2) are not obliterated and should be recorded carefully as they may be causally related to the hydropic state. The distribution of edema may itself suggest a diagnosis, such as the disproportionate amount of postnuchal fluid seen in monosomy X (Figs. 14.3 and 14.4), although it is not specific for this condition and may occur with other chromosome abnormalities or with normal chromosomes. The abdomen is often distended because of ascites (Fig. 14.5A) or hepatosplenomegaly (Fig. 14.6).

Internal

Complex congenital heart disease, pulmonary anomalies (Figs. 14.7 and 14.8) and intrathoracic masses (Figs. 14.9 to 14.13) are among the commonest associations of nonimmune fetal hydrops (see Tables 14.1 and 14.4) and should always be sought. Intraabdominal causes include tumors (Figs. 14.14 and 14.15), cystic kidneys, lower genitourinary obstruction, and intestinal pathology with hemorrhage or infarction (see Table 14.2).

The heart and liver are often enlarged even in the absence of specific abnormality in these organs. This is particularly likely when hydrops is due to heart failure. In rhesus disease and chronic anemia from other causes, including infection such as toxoplasmosis, hepatosplenomegaly is usual owing to increased, persistent hemopoiesis (Fig. 14.6). The liver is often much enlarged in the osteochondrodysplasias, as it is the main organ of erythropoiesis consequent upon reduced bone marrow volume.

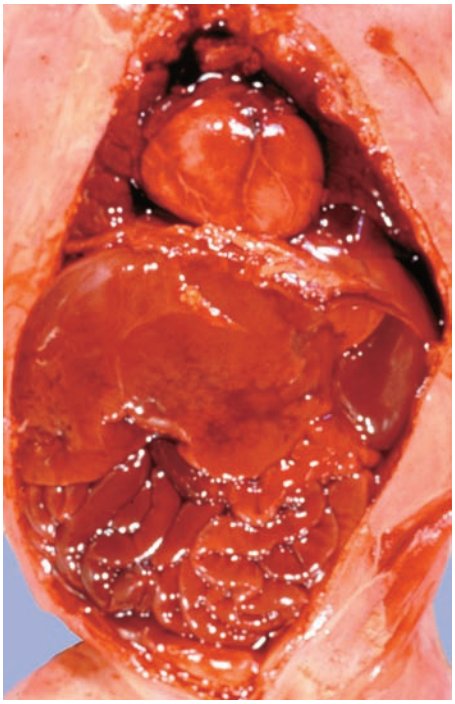


Figure 14.6. Fetal hydrops due to severe rhesus incompatibility. Massive hepatosplenomegaly is the result of increased erythropoiesis.

Figure 14.7. Hydropic macerated stillbirth: adenomatoid malformation of right upper lobe of lung. There is mediastinal shift and displacement of the liver by the depressed hemidiaphragm (Keeling 1994).

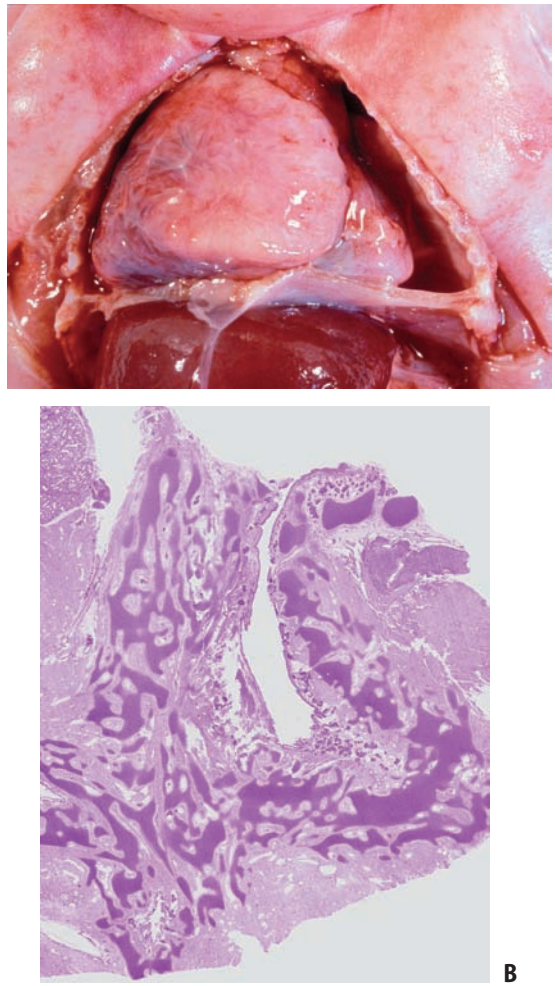


Figure 14.8. (A) A mesenchymal hamartoma replaces the right upper lobe of lung. (B) It comprised irregular bars of cartilage and myofibroblastic tissue.

Figure 14.9. Cardiac teratoma. The pericardial sac is markedly distended because of an effusion. A massive teratoma arises from the root of the heart. A few cysts are apparent on the cut surface.

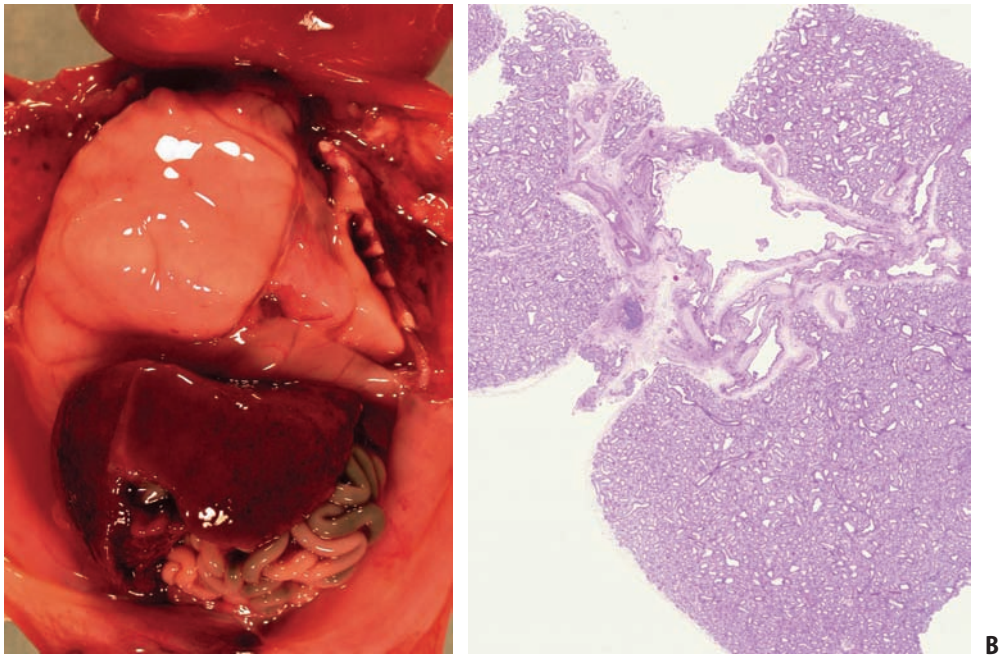


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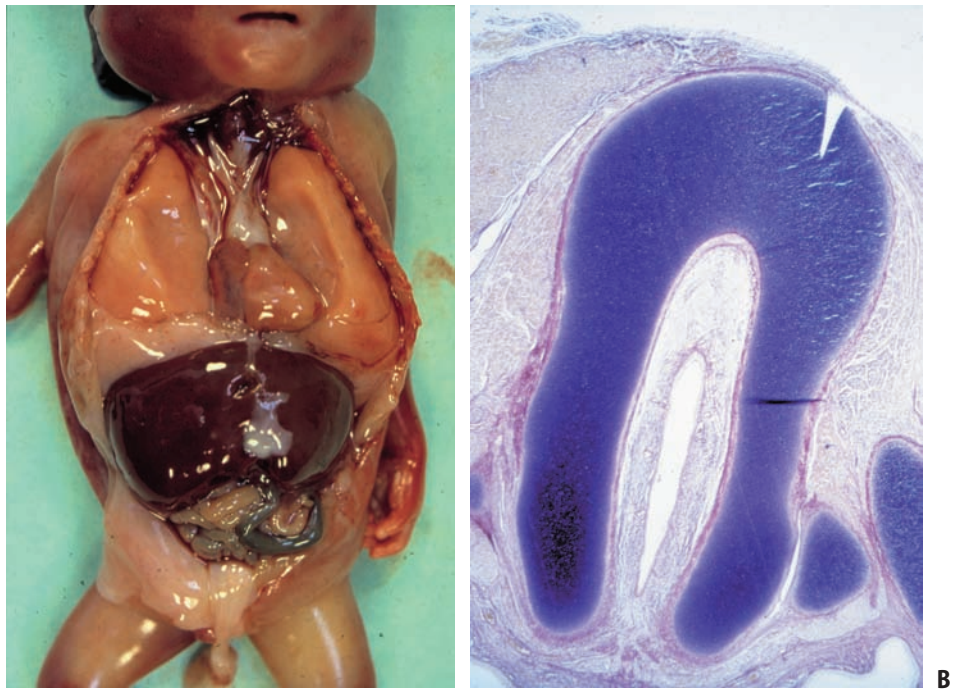
Figure 14.10. (A) Fetus with bronchial atresia affecting the right middle and lower lobar bronchi. There is expansion of the dependent lung parenchyma, resulting in mediastinal shift and hypoplasia of the left lung and right upper lobe. (B) The proximal bronchi are dilated and are devoid of cartilage in their walls. The affected lung tissue is hyperplastic.



A B

Figure 14.11. (A) Fetus of 20 weeks' gestation. There is massive distention of both lungs producing depression of the diaphragm, ascites, and hydrops. (B) Severe laryngeal stenosis because anomalous development of the cartilaginous skeleton reduces the lumen to a slit (Keeling 1994).

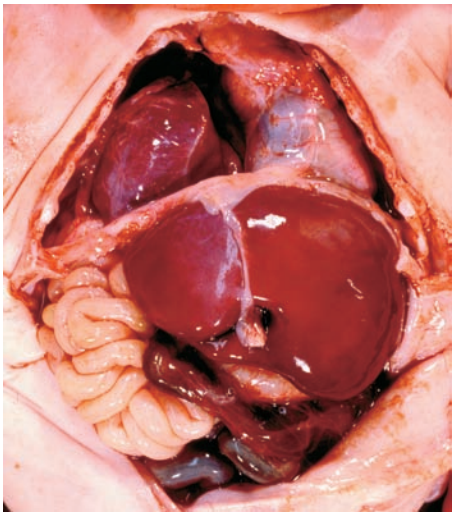


Figure 14.12. Right-sided diaphragmatic hernia associated with fetal hydrops and polyhydramnios. The heart and lungs are displaced to the left by the right lobe of the liver (Keeling et al. 1983).

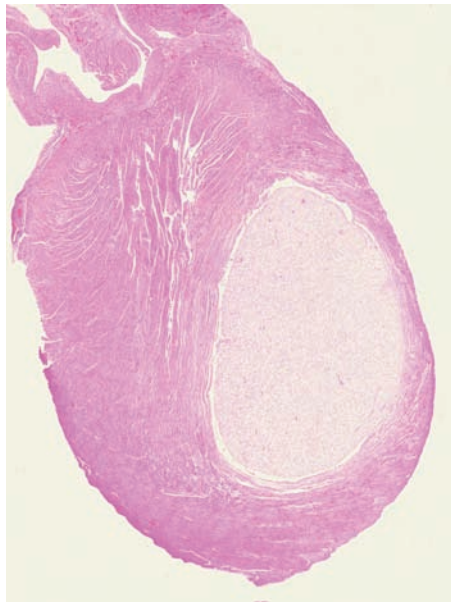


Figure 14.13. Rhabdomyoma of the cardiac interventricular septum. The tumor forms a well-circumscribed area within the myocardium.

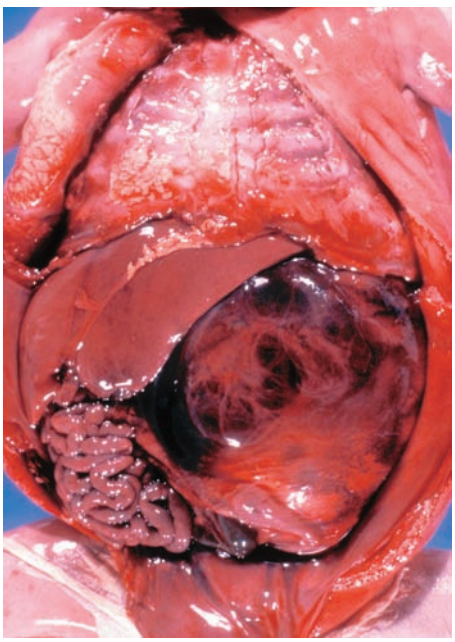


Figure 14.14. Congenital mesoblastic nephroma. The kidney tumor displaces the intestines and the liver. Hydrops has presumably resulted from obstruction of central venous return from the lower body. (Courtesy of Dr. E.S. Gray, Aberdeen, Scotland.)

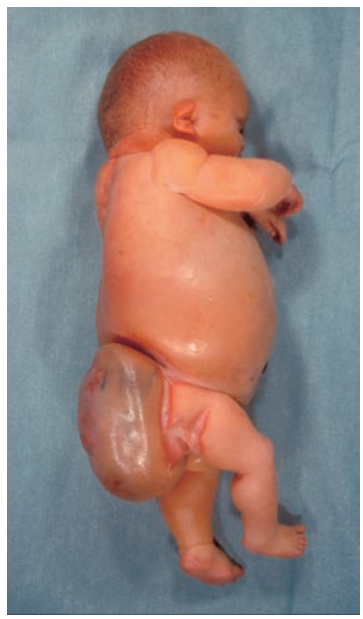


Figure 14.15. Hydropic neonate with sacrococcygeal teratoma. There was a large intrapelvic component that caused ureteral obstruction late in gestation.

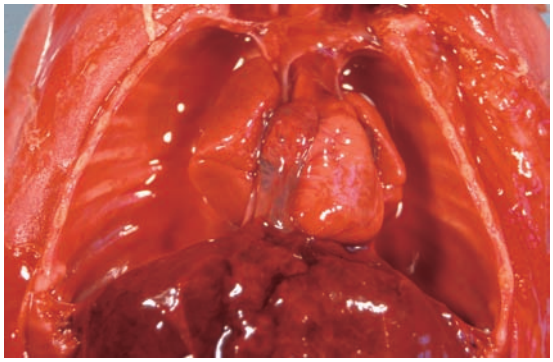


Figure 14.16. Large bilateral pleural effusions gave rise to severe pulmonary hypoplasia in this case.

Serous effusions are often present and are always found when hydrops is severe (Fig. 14.16). It is usual to find an excess of fluid in all body cavities, although not usually to the same degree. Sometimes there is disproportionate ascites, and it is likely that in some, if not many, of these babies, ascites accumulates first and hydrops follows because elevated intraabdominal pressure distorts the umbilical vein and impedes venous return from the placenta. In a review of fetal ascites, Machin (1985) found that many of the associations of fetal ascites were also associated with generalized hydrops, although there was an excess of intraabdominal abnormalities such as urinary tract obstruction and gastrointestinal or hepatic abnormalities. A very large pericardial effusion is unusual unless there is intrapericardial pathology such as teratoma (Bruch et al. 1997) (Fig. 14.9) or cardiac diverticulum (Prefumo et al. 2005).

Intracranial pathology includes necrosis and calcification resulting from infection, tumors, and vascular abnormalities (Fig. 14.17; see Table 14.3). Cerebral hemorrhage may be present.

Late disruptive defects, both intra- and extracranial, resulting from hypotensive ischemia or embolic phenomena should be sought in monochorionic twins (Weeks et al. 1996).

Histological Examination

Routine sampling of organs for histological examination should be undertaken (see Chapter 1) even when a gross abnormality thought to be causally related to the hydrops has been identified. Histological appearances of the heart and lungs may be particularly informative. The heart should be sampled initially in such a manner that

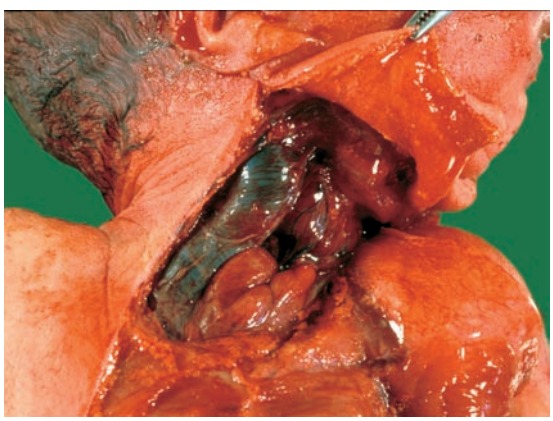


Figure 14.17. Hydrops was caused in this case by meningeal angiodysplasia, resulting in a state of excessively high cardiac output. Note the grossly enlarged jugular vein.

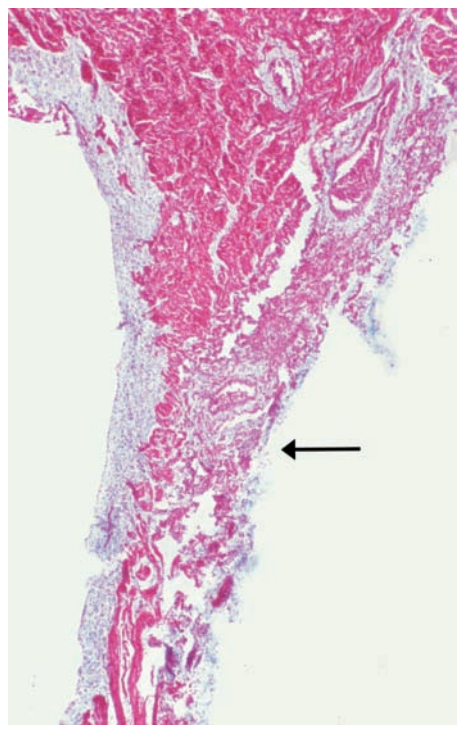


Figure 14.18. Female, 32 weeks' gestation: right atrial wall. The sinus node artery is clearly seen but the sinuatrial node is hypoplastic (arrow) (Masson's trichrome).

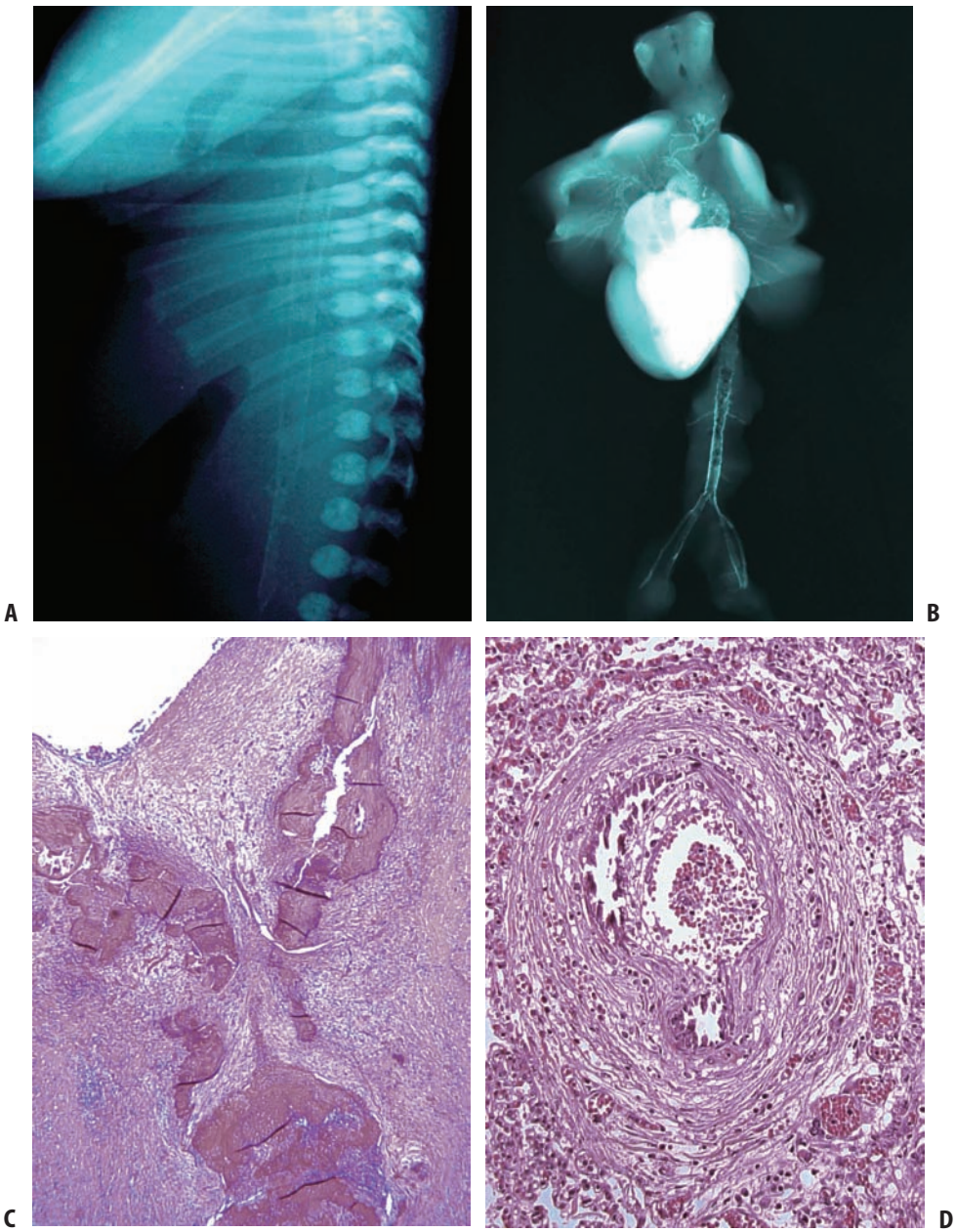


Figure 14.19. (A) Lateral radiograph of a hydropic stillbirth with diffuse calcific arteriopathy. The aorta is outlined on the film owing to excessive mineralization. (B) Postmortem radiograph of the dissected organ block from a similar case, clearly showing the calcification within the vessels, including the pulmonary, renal, and mesenteric arteries. (C) The proximal pulmonary arteries are heavily calcified. (D) More distal pulmonary artery branches show mineral deposits partially replacing the internal elastic lamina.

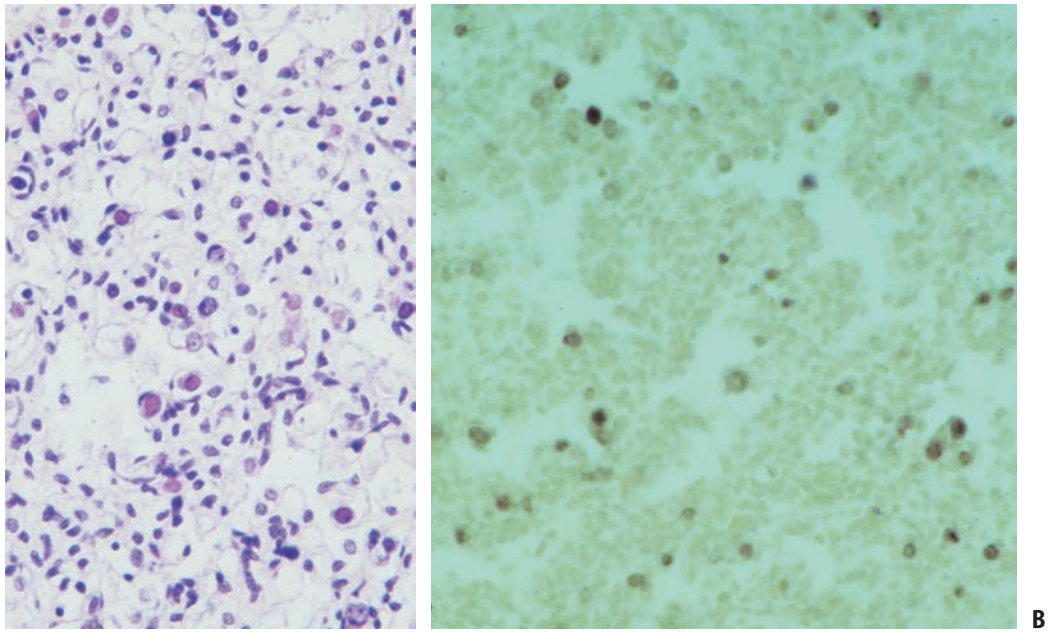


Figure 14.20. (A) Parvovirus infection. Intranuclear inclusions are present in cells in pulmonary capillaries. (B) In-situ hybridization demonstrates a large number of infected cells. (Courtesy of Dr. H. Porter, Leicester, England.)

the conduction system is undisturbed as subsequent systematic detailed examination may be necessary. In the myocardium, infection, infarction, or rhabdomyomata may be found. Histology (Fig. 14.18) will distinguish between different pulmonary hamartomata and between the various types of cystic kidney disease (see Chapter 22). The nature of vascular pathology can be established (Fig. 14.19). Useful information about the nature of a fetal infection can be obtained either from the histological appearances or from more specific investigations including immunohistochemistry or in situ hybridization (Fig. 14.20).

The Placenta

Fetal hydrops may be accompanied by edema of the placenta and umbilical cord, but the changes may not be uniformly distributed throughout the organ. The placenta is often extremely thick and may weigh more than 1000 g. It is pale and friable, (see Fig. 3.32 in Chapter 3) and there is usually marked edema of the umbilical cord. Placental appearance is related to the underlying cause of hydrops and is hydropic when there is severe anemia and when there is obstruction to venous return from the placenta. In congenital nephrotic syndrome, placental edema is gross even when the fetus is only mildly affected. The placenta may appear normal even when the fetus is severely hydropic due to high-output heart failure or some chromosome anomalies.

The placental abnormalities causally related to fetal hydrops are most frequently angiomata (Fig. 14.21) and monochorionic, diamniotic twin pregnancy. When there is significant twin-to-twin

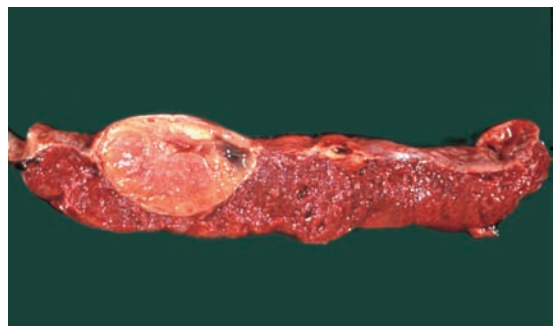


Figure 14.21. Slice of placenta with a large angioma from a pregnancy complicated by third trimester fetal hydrops and polyhydramnios.

transfusion, there may be a marked difference in appearance between the parts of the placenta related to each baby. The donor part is pale, friable, and often thicker than that related to the recipient, which is intensely congested. Vascular communications between the two circulations can be readily demonstrated in chorionic plate vessels, but it is within the placental parenchyma that significant (one-way) shunting takes place. Bajoria et al. (1995) draw attention to the paucity of chorionic plate anastomoses in symptomatic twin-to-twin transfusion. Monoamniotic twins, where anastomoses are very numerous, rarely exhibit signs of twin-to-twin transfusion syndrome (Fig. 14.22).

Histological examination of the placenta of a hydropic fetus reflects, to some extent, the gross appearance. When the placenta is hydropic, the villi may be uniformly edematous, for example congenital nephrotic syndrome, and Hofbauer cells are readily seen. In rhesus disease, villous hydrops is not usually gross and is patchy in distribution; many villi are of normal size. There is persistence of cytotrophoblast so that villi appear immature. Careful examination of the nonhydropic villi shows plugging of villous capillaries by clumps of erythropoietic cells (Fig. 14.23). In the fetus with neuroblastoma, similar capillary plugging by clumps of tumour cells is seen (Fig. 14.24). Prominence or increased size of trophoblast cells with cytoplasmic vacuolation is present in some fetuses with genetic metabolic disease such as the lysosomal storage disorder sialidosis (Fig. 14.25).

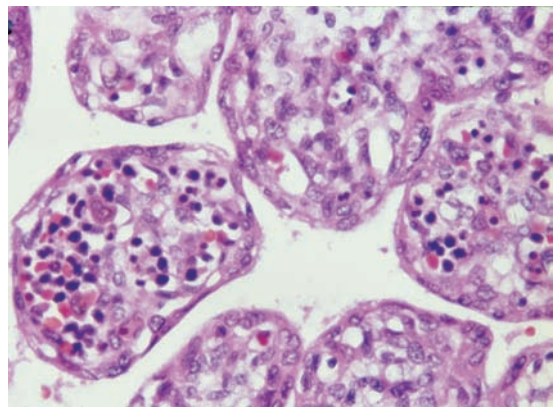


Figure 14.23. The placenta in rhesus hydrops. Hydropic change is nonuniform. Capillaries of nonhydropic villi are plugged by erythropoietic cells.

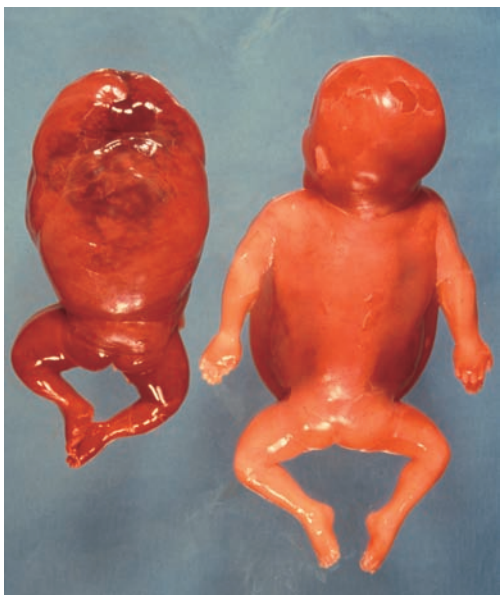


Figure 14.22. Acardiac twin. The acardiac twin (left) is plethoric, whereas the pump twin (right) is anemic and hydropic.

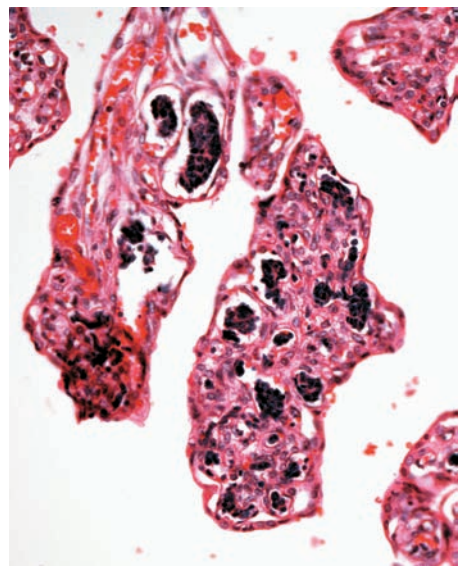


Figure 14.24. Hydropic fetus with neuroblastoma. Tumor emboli are present within villous capillaries in the placenta. (Courtesy of Dr. A. Siegal, Petach-Tikva, Israel).

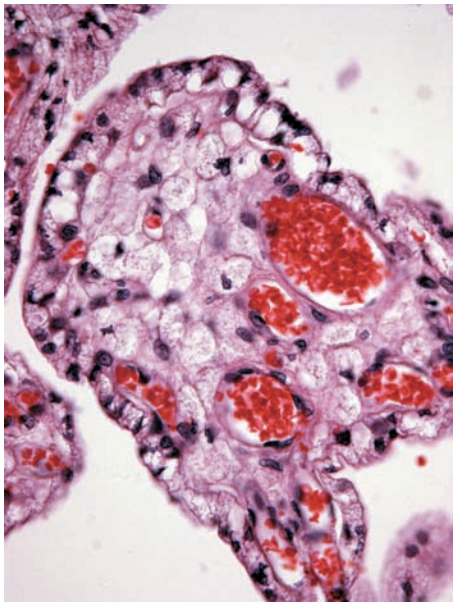


Figure 14.25. Placenta at 25 weeks' gestation. Prominent, vacuolated trophoblast and Hofbauer cells. Genetic metabolic disease (sialidosis).

Significance of Pathological Abnormalities

When assessing the relationship between fetal hydrops and any pathological abnormalities present, it is important that a causal relationship is not immediately implied and that thorough, systematic investigations are undertaken.

Careful thought must be given to all findings, clinical and pathological, so that the sequence of events culminating in fetal hydrops might be better understood. This is especially important as particular clinical skills are developed and effective fetal therapy is introduced to alleviate the hydropic state.

Mild subcutaneous edema is often observed when intrauterine death has occurred, and its significance is dubious. Intraperitoneal accumulation of fluid postmortem may be distinguished from significant ascites by its color, which is usually dark red, and the site and appearance of viscera. In the presence of long-standing ascites the intestines are compressed into the central posterior part of the peritoneal cavity, and pleural effusions not only cause some degree of pulmonary hypoplasia but also affect lobar contour, with loss of concavity of the inferior surface of the lower lobes and the acute angle of the inferior border.

McFadden and Taylor (1989) question the causality of association with both congenital heart disease and prenatal closure of the foramen ovale. They found no difference in the type of malformations seen in hydropic and nonhydropic babies subjected to necropsy examination. Looking at the types of cardiac malformation in series of 10 or more cases of hydrops (Keeling 1998), there seems to be an excess of complex cardiac anomalies, particularly those with ambiguous cardiac situs, where structural abnormalities of the cardiac conduction system and arrhythmias are common (Fleischer et al. 1981; Kleinman et al. 1982; Keeling et al. 1983).

Newbould et al. (1991) investigated the significance of endocardial fibroelastosis (EFE) in both rhesus and nonrhesus hydrops. They concluded that EFE was secondary to congestive cardiac failure. We endorse their conclusion that EFE is a response to insult rather than a primary disorder but find it difficult to ascribe the changes to congestive cardiac failure. It seems more likely that the common factor among possible antecedents is hypoxia. The presence of mature elastic and collagen fibers beneath the endothelium and the lack of cytological evidence of myohypertrophy suggest that in many cases congestive cardiac failure is a response to increased work in overcoming the impedance of EFE to normal myocyte contractility.

Intracranial ischemia (Kobori and Urich 1986; Squier and Keeling 1991) and intracranial hemorrhage (Bose 1978) require careful evaluation. Ischemic cerebral injury seems to be a consequence of the underlying cause of hydrops; Squier and Keeling (1991) found these changes in both the hydropic and nonhydropic twin of twin-transfusion pairs. One of these babies had an intracerebral hemorrhage that occurred in an area with preexisting ischemic change. Laneri et al. (1994) document a variety of fetal cerebral insults in nonimmunological hydrops from microcephaly and encephalomalacia to microcalcification, polymicrogyria, astrocytosis, and severe neuronal loss.

It is evident that careful consideration must be given to all pathological findings in the examination of the hydropic fetus. Assessment of histological changes may permit observations in respect to the chronology of changes present and contribute to our understanding of the mechanisms of fetal hydrops.

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15
Congenital Tumors

Adrian K. Charles

Tumors presenting in the newborn period are rare, although pathologists working at busy obstetric or neonatal units can expect to see occasional cases (Isaacs 1997, 2002b). The incidence is around 1 in 12,000 to 1 in 27,500 live births (Moore et al. 2003). Many of these tumors are specific to infants or behave differently from their counterparts in older children. Lack of familiarity with neonatal tumors may lead to unnecessarily aggressive therapy or well-intentioned neglect. Some neonatal tumors may appear to be aggressive lesions and yet be benign and, conversely, others look benign but may be fatal if incompletely excised. Most, but not all, childhood neoplasms have been described in the perinatal period. As in children generally, they are often mesenchymal rather than epithelial in histogenesis, and knowledge of normal human development is often useful. Space limitations prevent this chapter from being comprehensive, so the focus is on the special characteristics of neonatal tumors that influence their diagnosis and management, and this chapter also discusses some areas where the study of neonatal tumors is of interest to our understanding of neoplasia in general. Some characteristic lesions not mentioned elsewhere in the text are listed in Table 15.1. Isaacs (1997, 2002; Las Heras and Isaacs 1987) in particular has presented extensive reviews of the subject. Neonatal tumors accounted for 2.6% of all children's tumors in his series, of which 40% were malignant. About 40% of malignant tumors in neonates are evident on the first day of life, and 17% only discovered at autopsy (Campbell et al. 1987). Most malignant congenital tumors present in the first week.

A congenital tumor is one that is present at birth, but it is reasonable to suppose that any tumor presenting in the first 3 months of life is congenital. It is now becoming clear that other childhood tumors, including many leukemias, Wilms' tumors, bronchopulmonary blastomas, and neuroblastomas, appear to arise from cells or lesions that are already abnormal at the time of birth. Children who present with acute leukemia can be found to have identical genetic changes in their leukemia and in the DNA from their Guthrie card or in the leukemia in their monozygotic twin (Greaves 2005). More neonates have these genetic changes than do children who develop leukemia. These studies show that many childhood leukemias have precursor cells that have undertaken the initial genetic steps of neoplastic progression at birth, although they do not necessarily progress to malignancy, a situation well described with nephrogenic rests and Wilms' tumors. This then raises the question of why tumors in infants are different from those in adults, which may be partly explained by the time needed for mutations to develop in epithelial tissues for adult tumors to occur and for exposure to mutagenic environmental agents. In other cases, such as Wilms' tumors, the cell of origin is probably the metanephric blastema that regresses during development. However, for acute leukemia, for example, the stem cells persist through life and the reasons are less clear but probably relate to the fetal environment and development.

Table 15.1. Some lesions recognized in newborns, but not described elsewhere in the text

Anatomical site, tumor	Reference	Anatomical site, tumor	Reference
Head and neck		Heart	
Thymic cyst	Hendrickson et al. 1998	Cardiac fibroma	Burke et al. 1994
Mouth and nasopharynx		Rhabdomyoma	Webb et al. 1993
Gingival granular cell tumor (Fig. 15.1)	Ugras et al. 1997	Gastrointestinal	
Hairy polyp of the oropharynx (Fig. 15.3)	Kelly et al. 1996	Gastrointestinal stromal tumor	Bates et al. 2000
Nasal glioma	Madjidi and Couly 1993	Leiomyosarcoma	Kennedy et al. 1997
		Tailgut cyst	Antao et al. 2004
Nasopharyngeal brain heterotopia	Seibert et al. 1984	Pancreatoblastoma	Klimstra et al. 1995
Foregut duplication cyst of tongue	Rousseau et al. 2004	Nesidioblastosis and variants	See Chapter 24
		Gonads	
Hamartoma of the tongue	Owen et al. 1993	Congenital ovarian cysts	Antao et al. 2004
Sialoblastoma	Ortiz-Hidalgo et al. 2001	Juvenile granulosa cell tumor	Young et al. 1985
		Gonadoblastoid dysplasia	Spear and Martin 1986
Salivary gland anlage tumor	Dehner et al.		
	Herrmann et al. 2005	Cystic dysplasia of testis	Cho and Kosek 1985
		Testis adrenal rest with congenital adrenal hyperplasia (CAH)	Dogra et al. 2004
Skin and subcutis		Spine	
Neurocristic hamartoma	Pearson et al. 1996	Spinal hamartoma	Morris et al. 1998
Striated muscle hamartoma	Scrivener et al. 1998	Tails	James and Canty 1995
Smooth muscle hamartoma	Guillot et al. 1998		
Soft tissue		Bone	
Neuromuscular choristoma	Mitchell et al. 1995	Osteochondromyxoma of bone	Carney et al. 2001
Lung and thorax		Infantile cartilaginous hamartoma of the rib	Cohen et al. 1992
Rhabdomyomatosis of lung	Hardisson et al. 1997		
Pulmonary myofibroblastic tumor	Alobeid et al. 1997	Brain	
Massive mesenchymal malformation of lung	Khong and Keeling 1990	Hypothalamic hamartoblastoma	Squires et al. 1995
		Miscellaneous	
		Accessory scrotum	Amann et al. 1996

Although there is no absolute distinction between the histological types of tumors presenting at birth and in early infancy, there are important clinical differences that make the distinction worth preserving. For example, tumors are now not infrequently diagnosed in utero by the anatomy scan in the second trimester. This helps management of the pregnancy and delivery, and novel approaches such as the ex utero intrapartum treatment (EXIT) procedure have been introduced (Shih et al. 2005). Large tumors can rupture or obstruct delivery or give rise to fetal hydrops, if vascular, or affect the fetal cardiovascular system. The fetal circulation may be responsible for particular patterns of metastasis seen in the neonate. The outcome often depends more on the size and site of the lesion than on the histology.

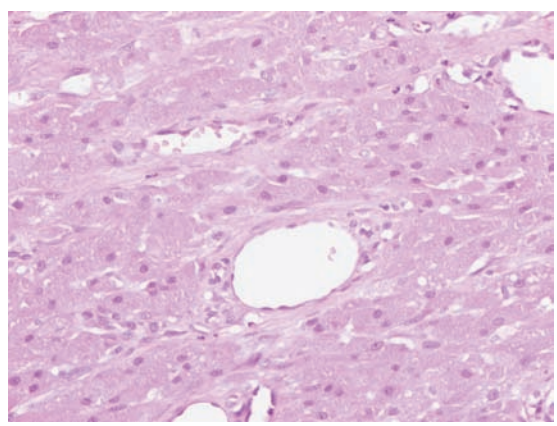

Figure 15.1. Granular cell epulis/congenital granular cell tumour. Typical presentation with a female neonate with a 2-cm mass arising from gingival margin.

Reduced tolerance to drugs and especially radiation may complicate therapy in very young babies (Meadows et al. 1992; Weitzman and Grant 1997).

Many of the tumors seen in the newborn are hamartomas, though the distinction between neoplasm, hamartoma, and choristoma is often unclear, and may be semantic in some cases. It is difficult to make a comprehensive classification system for these tumors; some segregate according to histological type and others according to the usual site of presentation. Some of the true neoplasms of childhood are collectively referred to as blastomas or embryonic tumors. These include nephroblastoma (Wilms' tumor), neuroblastoma, retinoblastoma, hepatoblastoma, medulloblastoma, pleuropulmonary blastoma, and embryonal rhabdomyosarcoma. These tumors tend to recapitulate embryonic tissues and are thought to arise from genetic changes in immature tissue, or persistent fetal stem cells. This explains their unique histology and restricted age range. Premalignant, persistent fetal lesions are exemplified by in-situ neuroblastoma (Beckwith and Perrin 1963) and nephrogenic rests (Beckwith et al. 1990; Beckwith 1993). Their frequent associations with malformations as discussed below further demonstrate the developmental origin of some childhood tumors.

Incidence

Benign tumors of the newborn are common, and many are not formally recorded. Vascular nevi and hemangiomas are present in 6% to 25% of the pediatric population, most being congenital, although they often present after birth. Strawberry hemangiomas are more common in very low birth weight babies than in controls (Amir et al. 1986). While most strawberry hemangiomas and many visceral hemangiomas regress, even benign hemangiomas may cause death, for example, by causing heart failure or a consumptive coagulopathy. Melanocytic nevi are found in 3% of newborn white infants and 16% of nonwhite infants in contrast to the extreme rarity of congenital malignant melanoma (Koyama et al. 1996).

It is difficult to estimate the incidence of malignant congenital tumors from the literature. Most series are not population based, and many are not comparable because they include different age ranges (Table 15.2). Series extend over many years, during which the treatment and classification of tumors has changed.

Teratomas are the commonest reported neonatal tumor but neuroblastoma is the commonest malignant tumor followed by leukemias (which is the commonest fatal congenital tumor)

Table 15.2. Benign and malignant tumors in newborn children and infants (percentage by tumor type)

Tumor	Neonates (%) (Las Heras and Isaacs 1987)	Infants up to 1 year (%) (Isaacs 1983)	Malignant tumors*; infants up to 1 year (%) (Birch and Blair 1992)	Malignant tumors*; neonates (%) (Campbell et al. 1987)	Perinatal tumors (%) (Isaacs 2002)
Teratoma	40	18	3		25
Neuroblastoma	12	13	22	47	26
Mesenchymal	9	23	9	12	9.6
Renal	5	7	13	4	4.9
Central nervous system	2	9	18	9	10.4
Leukemia	12	6	20	8	12.2
Number of cases	42	265	92	102	607

*Retinoblastoma accounted for 17% of the malignant tumors in these series.

and mesenchymal tumors of various types, renal tumors, and brain tumors (Moore et al. 2003; Vasilatou-Kosmidis 2003). Less common conditions seen in the neonatal period include Langerhans' cell histiocytosis, hepatoblastoma, and retinoblastoma. Lymphoma, clear cell sarcoma of the kidney, and anaplastic Wilms' tumor are notable for their extreme rarity in very young infants. A 60-year series from Canada showed neonatal (up to 29 days) malignant tumors to comprise 2% of pediatric malignancies and to have a mortality rate of 41%, predominantly due to neuroblastoma (Campbell et al. 1987). Neuroblastoma was also the commonest tumor in a review of malignancy in neonates at St Jude Children's Research Hospital from 1962 to 1988 (Crom et al. 1989).

A study of 17,417 perinatal necropsies carried out in Melbourne over five decades found 46 congenital tumors, which included 24 teratomas, most frequently of the head and neck, followed by sacrococcygeal and mediastinal teratomas (Werb et al. 1992). Vascular tumors, neuroblastoma, and cardiac rhabdomyoma were next in frequency. Twenty percent of the affected babies had developmental anomalies, mainly associated with teratomas. Some babies presented with maternal polyhydramnios or fetal hydrops, most often with teratoma. Figures derived from biopsy records probably underestimate the incidence of congenital leukemia (Las Heras and Isaacs 1987). With that exception, they are essentially similar to data from the United Kingdom Children's Cancer Study Group and the Oxford Childhood Cancer Research Group registry (Palmer and Williams 2005). The Third United States National Cancer Survey (1969–1971) estimated the incidence of malignant neoplasms in infants less than 29 days of age as 36.5 per million live births per year (Bader and Miller 1979). Mortality was approximately one fifth of the incidence, reflecting the relatively benign course of several histologically malignant tumors in this age group. Pathologists should be aware that standard histological criteria of malignancy such as high mitotic rate, immature cells, necrosis, and even vascular invasion do not always indicate malignant behavior in congenital tumors. In a series from Manchester, germ cell tumors were most frequent. The incidence of malignant tumors in infants up to 1 year was 121 per 10^6 children of ages 0 to 14 years (Birch and Blair 1992). A large population-based study of infants up to 1 month old in the West Midlands from 1960 to 1989 showed an incidence of benign and malignant neonatal tumors of 0.07 per 1000 live births per year, also with a predominance of teratoma (mostly benign) followed by neuroblastoma and leukemia. The 5-year survival rate was 50% (Parkes et al. 1994). Congenital tumors were associated with polyhydramnios, which was not specific to any particular tumor type. Fifteen percent of patients had some congenital anomaly.

Etiology

Congenital tumors appear to offer a system in which to study oncogenesis free from the multiple environmental influences that complicate such studies in adults. However, the sperm, egg, embryo, and fetus are exposed to many chemical, physical, and infective agents in utero, and the intrauterine environment can alter the risk of infant and childhood neoplasia (Knox 2005).

In recent years considerable insight has been gained into the pathogenesis of neoplasms in infants and young children, and the molecular pathogenetic pathway is beginning to be understood. Genetic accidents are part and parcel of human mitotic activity. The large number and rapidity of cell cycles required during embryonic and fetal growth provide ample opportunity for such mistakes. The genetic mechanisms involved in oncogenesis, which include small mutations, loss of heterozygosity, and changes in genomic imprinting, are being shown to involve genes that normally regulate the cell cycle, apoptosis, and development (Knudson 2002; Scotting et al. 2005). Genetic, chromosomal, syndromic, and environmental associations that have been recognized in childhood cancer are discussed here as they apply to perinatal pathology (Moore et al. 2003).

Table 15.3. Some inherited syndromes associated with childhood cancer

Inherited syndrome	Childhood cancer
Phakomatoses and hamartoses	
Neurofibromatosis type 1	Brain tumors; sarcomas; leukemia, carcinoid
Tuberous sclerosis	Brain tumors
Basal cell nevus syndrome	Medulloblastoma; basal cell carcinoma
Turcot's syndrome	Medulloblastoma
Multiple mucosal neuroma syndrome	Medullary thyroid carcinoma; pheochromocytoma
Metabolic disorders	
Glycogenosis type 1	Hepatocellular carcinoma
Hereditary tyrosinemia	Hepatocellular carcinoma
α1-antitrypsin deficiency	Hepatocellular carcinoma
Chromosome breakage and repair defects	
Bloom's syndrome	Leukemia; gastrointestinal tumors
Ataxia telangiectasia	Leukemia; lymphoma
Fanconi's anemia	Leukemia; hepatoma
Xeroderma pigmentosum	Skin cancers; melanoma
Immune deficiency disorders	
Wiskott-Aldrich syndrome	Leukemia; lymphoma (often in CNS)
Sex-linked lymphoproliferative syndrome	B-cell lymphoma
Severe combined immunodeficiency	Leukemia; lymphoma
Bruton's agammaglobulinemia	Leukemia; lymphoma
Rothmund-Thompson syndrome	Osteosarcoma
Noonan syndrome	Juvenile myelomonocytic leukemia, giant cell tumors
Rubenstein-Taybi syndrome	CNS tumors, rhabdomyosarcoma, leukemia
Carneys complex	Myxomas, schwannomas, osteochondromyxoma, Sertoli cell tumors

CNS, central nervous system.

Inherited Tumors

Some childhood tumors are inherited. For example, 40% of retinoblastomas and a small proportion of Wilms' tumors are familial (Pastore et al. 1988; Haber and Housman 1991; Narod et al. 1991). Nine percent of retinoblastomas are present at birth, and these are almost always heritable and attributable to a mutation of the retinoblastoma gene on chromosome 13q. Sibships affected by neuroblastoma, teratoma, hepatoblastoma, or congenital fibromatoses have all been reported, but the genes responsible are largely unknown. Many inherited syndromes predispose to tumor development (Table 15.3), although such tumors are not usually present at birth (Bolande 1976; Pritchard-Jones 1996; Lynch et al. 1997; Knudson 2002).

Hereditary tumors account for only a small proportion of childhood cancers; Narod et al. (1991) estimates at least 4% are due to inherited genetic factors.

Malformation Syndromes and Tumors

The association of trisomy 21 and leukemias is well known (see later), though children with Down syndrome may have a lower rate of solid tumors than those with a normal karyotype (Hasle et al. 2000). A further group of patients in this study had leukemia and constitutional aneuploidy, mainly trisomy 21. Neonatal tumors are reported with trisomies 13 and 18, the latter particularly with nephroblastoma and hepatoblastoma (Bove et al. 1996; Satge and Van Den Berghe 1996). The association of constitutional karyotypic abnormalities and childhood cancer can be helpful in localizing key genes involved in particular tumor oncogenesis (Table 15.4). The most

Table 15.4. Constitutional chromosomal anomalies predisposing to childhood cancer

Chromosome anomaly	Childhood cancer
Down syndrome (trisomy 21)	Acute leukemia
Turner syndrome (45 XO)	Neurogenic tumors
13q-syndrome	Retinoblastoma
11p-syndrome	Nephroblastoma
Monosomy 7	Preleukemia and nonlymphoblastic leukemia
XY gonadal dysgenesis	Gonadoblastoma
Trisomy 18	Nephroblastoma
Variegated mosaic aneuploidy	Rhabdomyosarcoma, Wilms' tumor, leukemia
Klinefelter syndrome	Leukemia; teratoma; breast carcinoma

frequent associations likely to be seen by perinatal pathologists are nephrogenic rests in trisomy 18 and congenital leukemia associated with trisomy 21.

Several dysmorphic syndromes and malformations carry significant risk of childhood cancer (Nishi et al. 2000) (Table 15.5). The best known are hemihypertrophy and Beckwith-Wiedemann syndrome (BWS). Ten percent to 21% of children with BWS develop neoplasms (Weksberg and Squire 1996), and the different genetic subtypes of BWS have different pheotypes and risk of malignancy (Weksberg et al. 2001; DeBaun et al. 2002). The occasional occurrence of neuroblastoma in children with Hirschsprung's disease and Ondine's curse suggests that it may sometimes be part of a generalized disorder of neural crest development (Stovroff et al. 1995). The overlapping molecular pathology of tumors and malformations are seen in the different conditions associated with *RET* gene mutations, from Hirschsprung's disease to multiple endocrine neoplasia type 2 (MEN-2) syndromes and thyroid cancer.

Table 15.5. Some congenital anomalies and malformation syndromes associated with childhood cancer

Congenital anomaly	Childhood cancer
Hemihypertrophy and Beckwith	Nephroblastoma; syndrome, adrenal cortical tumors, hepatoblastoma
Sporadic aniridia (WAGR syndrome)	Wilms' tumor
Poland syndrome	Leukemia
Hirschsprung's disease	Neuroblastoma
Denys-Drash syndrome	Wilms tumor
Perlman syndrome	Wilms tumor
Gorlin syndrome	Desmoplastic medulloblastoma, soft tissue rhabdomyoma

Nonsyndromic Malformations and Tumors

The association of nonsyndromic malformations and childhood tumors is less clear. Some authors describe an increased incidence of congenital malformations in children with cancer, but methods of ascertainment and inclusion of minor malformations may bias their results. A retrospective review of the case records of nearly 400 children with embryonic tumors showed no excess of major malformations when those discovered because of the tumor or possibly attributable to it were discounted (Berry et al. 1970). Nevertheless, associations between Wilms' tumor and genitourinary abnormalities, and between sacrococcygeal teratoma and hindgut, sacral, and genitourinary malformations, are firmly established, and may be due to an underlying common genetic anomaly. One such association is the Currarino syndrome [On-Line Mendelian Inheritance in Man (OMIM) 176450] due to mutations of the homeobox gene *HLXB9*, though the teratomas associated with this syndrome appear almost always to have a benign prognosis (Weinberg 2000). Slow-growing, long-standing fetal tumors can disrupt normal fetal development, as seen in sacrococcygeal teratomas. Most malformations are not associated with cancers in the parents, though achondroplasia and cleft palate may be exceptions (Zhu et al. 2002; Stoll and Feingold 2004).

The association of malformations and tumors is complex because there are different mechanisms underlying the association. In some cases, such as congenital cystic adenomatoid malformation, the anomaly appears to increase the risk of an adenocarcinoma developing. In BWS the same genetic changes that cause the anomaly and organ hyperplasia defect are also important in oncogenesis

(Weksberg et al. 2001). In the WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), the constitutional loss of the contiguous genes *PAX6* and *WT1* gives rise to the aniridia and the Wilms' tumor, respectively (OMIM 194072). Diethylstilbestrol gives rise to abnormal development that is prone to secondary malignant change (Herbst et al. 1971; Herbst 1999). In the variegated aneuploidy syndrome, the underlying genetic instability gives rise to the malformations and the tumors (OMIM 257300).

A review of the records of 20,304 children with cancer in Britain from 1971 to 1986 found the frequency of anomalies to be 4.4% in children with solid tumors and only 2.6% in those with leukemia/lymphoma (Narod et al. 1997). The cancers most frequently associated with anomalies were Wilms' tumor (8.1%), Ewing's sarcoma (5.8%), hepatoblastoma (6.4%), and gonadal and germ cell tumors (6.4%). The rate of malignant tumors in low birth weight children and twins is close to that of the general population (Windham et al. 1985), although very low birth weight infants are predisposed to hepatoblastoma (Ikeda et al. 1997; Tanimura et al. 1998; Ansell et al. 2005). Twins had a higher rate of renal tumors in one study (Windham et al. 1985). Another showed an increased risk of tumors with increasing birth weight, especially for cancers diagnosed before 2 years of age (Yeazel et al. 1997).

In a study of major birth defects associated with childhood cancer in Georgia and Iowa, pyloric stenosis was associated with a seven times risk of cancer in Georgia (Mili et al. 1993a), but not in Iowa (Mili et al. 1993b). The well-known association of Down syndrome with leukemia was observed, as was an association of birth defects with brain tumors and neuroblastoma. Other reports describe malformations in children with rhabdomyosarcoma (Ruymann et al. 1988), Langerhans' cell histiocytosis (Sheils and Dover 1989), Wilms' tumor, and neuroblastoma (Nakissa et al. 1985).

Environmental Agents, Maternal Medical Therapies, and Tumors

There is good experimental evidence that teratogenesis and oncogenesis are closely linked. Agents that induce tumors postnatally may produce malformations when given earlier in gestation (Bolande 1999; Arnon et al. 2001). There are three phases of risk: the pre- and periimplantation period, the organogenesis phase, and the fetal phase. The first phase is resistant to teratogenesis, but may abort; the second phase is sensitive to teratogens; and the third phase is more resistant to teratogenesis (Arnon et al. 2001). Experimental studies show the organogenesis phase of development is relatively resistant to tumor development, but this increases after organ formation (Bolande 1999). Ionizing radiation shows a similar gestational age-related effect (Einhorn 1983). The fetus usually tolerates maternal chemotherapy in pregnancy (Gwyn 2005). Irradiation for maternal malignant disease does cause some fetal damage, depending on the gestational age and the amount of irradiation, but is not necessarily dire (Kal and Struikmans 2005). It increases the risk of childhood leukemia by around 40% (Arnon et al. 2001). Immature animals are more susceptible to mutagens than older animals (Anderson et al. 1991).

There are many examples of exogenous teratogens in humans (Berry 1996), and some evidence that intrauterine exposure to chemical agents induces tumors in humans (Miller 1978; Perera 2000). Anecdotal reports describe nephroblastomatosis after in utero aspirin intoxication (Bove et al. 1979), mesothelioma in a child exposed to isoniazid prenatally (Tuman et al. 1980), and neuroblastoma in fetal hydantoin syndrome (Allen et al. 1980) and after exposure to maternal carbamazepine (Baptista et al. 1998). The association of maternal drug use and neonatal tumors is discussed by Satge et al. (1998a) and Arnon et al. (2001). A review of U.S. studies of pediatric tumor epidemiology showed few environmental risk factors for pediatric tumors, though there are some reports of increased risk of pediatric tumors (Wilms' tumor and hepatoblastoma) with paternal occupational exposure to hydrocarbons, suggesting a prezygotic influence and possibly pesticide exposure with acute myeloblastic leukemia (Buckley 1992).

The association of maternal ingestion of diethylstilboestrol during pregnancy with genital tract malformations and subsequent development of clear-cell adenocarcinoma of the vagina and cervix is well known (Herbst et al. 1971). Maternal use of neurotropic drugs, alcohol, and hair dyes during pregnancy has been associated with neuroblastoma (Terracini et al. 1983; Kramer et al. 1987). Paternal exposure to chemicals is associated with brain tumors (Terracini et al. 1983; Wilkins and Sinks 1990) and renal tumors (Fear et al. 1998). The risk of childhood leukemia/lymphoma and Wilms' tumor was related to maternal smoking during pregnancy in a case-control study and, although any effect was small, it was related to the number of cigarettes smoked (Stjernfeldt et al. 1986). Shu et al. (1996) found maternal alcohol intake, but not smoking, increased the risk of infant leukemia. A United Kingdom series has not found good evidence of an association of childhood cancer and parental smoking (Pang et al. 2003). Cell culture models have suggested a mechanism of genetic mutations, such as for the insecticide Permethrin and *11q23/MLL* gene changes (Borkhardt et al. 2003).

Children exposed in utero to atomic bomb radiation at Hiroshima in 1945 had an increased incidence of congenital malformations but not of cancers, while those exposed after birth developed more tumors (Jablon and Kato 1970). It is now accepted that prenatal radiation predisposes to childhood cancer (Harvey et al. 1985; Doll and Wakeford 1997). The Chernobyl nuclear power plant incident in 1986 has demonstrated that fetal and infant thyroid is more sensitive than adult thyroid to radiation (Williams 1993). *RET* gene mutations are usually involved in both follicular and papillary thyroid tumors following radiation (Bounacer et al. 1997). The debate about overhead power lines and childhood cancer is still ongoing, though the evidence at the moment is that this risk is, at most, small, and studies have led to statistical arguments (Draper et al. 2005). Paternal irradiation and the risk of leukemia and other tumors in their children has been a topic of serious debate, but any effect appears to be very small (Hicks et al. 1984; Doll et al. 1994; Draper et al. 1997). There is no increased risk of non hereditary tumors in the offspring of long-term survivors of childhood cancer (Sankila et al. 1998), although female survivors of Wilms' tumor who received abdominal irradiation have an increased rate of spontaneous abortion, perinatal death, and lighter babies than nonirradiated survivors (Green et al. 1982; Hawkins and Smith 1989). Antenatal ultrasound scanning does not cause tumors (Reece et al. 1990). Maternal viral infections have been linked with subsequent development of cancer in children (Nigro et al. 1993). There is also growing evidence that an infection may be needed for the "second hit" to allow progression of already genetically abnormal cells to progress to leukemia (Greaves 2005).

There is evidence that in vitro fertilization pregnancies have an increased risk of imprinting abnormalities including BWS (Shiota and Yamada 2005). The increasing incidence of undescended testis, hypospadias, and germ cell tumors of the testis (testicular dysgenesis syndrome) appears to be due to environmental changes (Skakkebaek et al. 2001).

Folate may have a protective effect for childhood acute leukemia as well as the reduction of neural tube defects (Thompson et al. 2001).

Oncogenesis

Knudson (1971) proposed a two-hit theory of carcinogenesis. From studies of retinoblastoma he postulated that two separate random and rate-limiting genetic events or "hits" are required to transform a cell and produce a tumor. In patients with hereditary retinoblastoma the first "hit" has already affected the genome, thus explaining the predisposition of these children to develop retinoblastomas that often arise early and may be multifocal. Knudson and Strong (1972) and Knudson and Meadows (1976) later included Wilms' tumor and neuroblastoma in their two-hit hypothesis. The hypothesis does not explain all the observed facts but it has had a profound effect on theoretical oncology (Haber and Housman 1991; Wheldon et al. 1992). The various dysmorphic syndromes, focal tissue abnormalities, chromosomal anomalies, and inherited disorders predisposing to childhood cancers may be evidence that the first genetic event has already occurred and that the child (or at least cells

within the child) are vulnerable to any subsequent "hit" or oncogenic event. In retinoblastoma, evidence of the first hit may be a positive family history or the 13q– phenotype. For Wilms' tumor the visible evidence may be nephroblastomatosis, hemihypertrophy, BWS, or aniridia. The development of both retinoblastoma and nephroblastoma is associated with further genetic events, resulting in loss of heterozygosity for regions of chromosome 13 and 11, respectively. These regions contain genes involved in control of normal growth and development of the retina and kidney. Although it is not their usual function, these genes have been called tumor suppressor genes because their homozygous loss or mutation results in tumors.

The genetic mechanisms involved in the majority of tumors are more complex than two hits of a tumor suppressor gene. Oncogenes, growth promoter, cell cycle regulator, apoptosis-related, and differentiation genes are all involved. The genetic mechanisms include loss of function, increased function, gene regulatory (promoter and suppressor coding regions), methylation, and probably noncoding RNA changes as well as translocations that either affect gene regulation or give rise to novel genes [e.g., the t(12;15) in mesoblastic nephroma] (Knezevich et al. 1998). These translocations are very helpful diagnostically but not always tumor-specific; t(12;15) is also seen in a type of breast cancer (Makretsov et al. 2004). We are ignorant of the molecular mechanisms responsible for the phenotype of most malformations and dysmorphic syndromes. The BWS promises to throw light on the relationship among dysmorphic syndromes, genes, and cancer. The segment involved, 11p15, embraces a cluster of imprinted genes and includes insulin-like growth factor-2 (IGF-2), *H19*, and cyclin-dependent kinase inhibitor-1C (CDKN1C) *($p57^{KIP2}$)* (Reik and Maher 1997), offering possible explanations for the abnormalities of growth, carbohydrate metabolism, and tumor susceptibility seen in this syndrome. Many individuals with the syndrome show paternal trisomy or isodisomy for this region of 11p15 and overexpression of IGF-2, but the syndrome is genetically and phenotypically heterogeneous (Weksberg et al. 2001; DeBaun et al. 2002).

Table 15.6. Some tumors of early life showing benign tendencies

Nephrogenic rests
Neuroblastoma:
In situ neuroblastoma
Stage IV-S neuroblastoma
Neuroblastoma under 1 year of age
Congenital myeloproliferative disorder in trisomy 21
Congenital fibromatosis (myofibromatosis)
Congenital fibrosarcoma
Yolk sac tumor of testis under 2 years of age
Hereditary retinoblastoma (retinomas)

Investigation of Congenital Tumors

In stillbirths, it is important to establish whether the tumor is part of a syndrome with genetic implications for subsequent pregnancies or a sporadic condition. This requires a full necropsy, including examining the placenta and retaining samples of both tumor and nontumor tissue for cytogenetics and molecular studies. A surgical specimen requires similar considerations. An accurate tumor diagnosis is needed, and the possibility of a syndromic cause should be considered. The management of these tumors is dealt with in general texts, but retention of appropriate samples for electron microscopy and for cytogenetic and molecular studies from tumor and nontumor tissue may be needed. Histological markers with important implications such as adrenal cytomegaly, pancreatic islet-cell hyperplasia, and nephroblastomatosis should be sought in both postmortem and surgical material.

Many histologically malignant-appearing tumors behave in a relatively benign way in neonates (Table 15.6) (Bolande 1976). Advice must be based on knowledge of the behavior of the tumor in children of the same age (Batcup 1992).

Teratomas

Teratoma has been the subject of numerous reviews (Grosfeld et al. 1976; Marsden et al. 1981; Tapper and Lack 1983; Lack et al. 1985a,b; Kooijman 1988; Perlman and Kretschmar 1997; Heerema-McKenney et al. 2005). In infancy, most are extragonadal. Sacrococcygeal teratomas outnumber all others, making up approximately 50%

to 70% of teratomas in this age group. The origin of teratomas is controversial, and it is likely that not all arise in the same way. Chromosome markers show that sacrococcygeal teratomas arise from totipotent somatic cells and are of the host sex. Some are clonal (Sinnock et al. 1996), and there may be some chromosomal changes (Veltman et al. 2005). Extragonadal and testicular germ cell tumors appear to arise from either totipotent somatic cells or premeiotic germ cells (Hoffner et al. 1994). Isochromosome 12, seen in many germ cell tumors in older children and adults, is not seen in teratomas of the newborn, while neonatal germ cell tumors may show 1p and 6q deletions and translocations involving 12q13, again suggesting they are biologically different (Veltman et al. 2003).

Most congenital teratomas fulfill the usual diagnostic criteria and contain tissues from all three germ layers, although about one quarter do not. Highly differentiated teratomas with limbs and organoid development may have features of a parasitic twin or fetus in fetu. Teratomas have been defined as being different from fetal development as they lack a notochordal or vertebral axis and have an inherent tendency toward progressive uncoordinated growth. However, teratoma and fetus in fetu have been reported occurring together (Du Plessis et al. 1974), and the distinction between these two entities is not as clear-cut as has been suggested (de Lagausie et al. 1997; Hopkins et al. 1997). Intracranial teratomas with multiple "fetuses" have been described (Naudin ten Cate et al. 1995). It is likely that the difference reflects the degree of linear early embryonic differentiation of the tumor.

The most important prognostic factors in teratomas of the newborn period (age is an important factor) are presence or absence of a yolk sac component, site, and completeness of excision. Immature neural elements are not malignant. Besides yolk sac elements, Wilms' tumors are seen as part of a malignant teratoma occasionally, whereas choriocarcinomas and germinomas appear vanishingly rare in this entity (Ward and Dehner 1974). Other immature elements should be related to the maturity of the host. Even teratomas with a high degree of organoid differentiation occasionally recur. Overall, around 10% of congenital teratomas are malignant (Isaacs 2002b).

Sacrococcygeal Teratomas

Sacrococcygeal teratomas occur in 1 in 30,000 to 1 in 170,000 live births, with a male to female ratio of 1:3 (Noseworthy et al. 1981; Bale 1984; Perlman 1998; Sebire et al. 2004). Most sacrococcygeal teratomas are sporadic but familial cases occur. A strong family history of twinning is described. Associated malformations of the hindgut, caudal spinal cord, and distal genitourinary tract are often present. Lemire and Beckwith (1982) and Bale (1984) described an extraordinary variety of developmental abnormalities and tumor-like conditions of the sacrococcygeal region in children and related them to the complex embryology of this area, which probably underlies the predilection of this site in neonates for teratoma. The Currarino association has been mentioned above.

Most tumors are apparent at birth and some are now diagnosed at the time of the antenatal anatomy scan, though it is likely that many will be undetectable in the second trimester. Sacrococcygeal teratomas are a cause of raised maternal serum α-fetoprotein (Chisholm et al. 1998). Management includes planned delivery in a center with expert pediatric surgery. Unfavorable factors are presentation before 30 weeks' gestation, rapid tumor growth, and the development of fetal hydrops or placentomegaly (Flake et al. 1986; Chisholm et al. 1998). Fetal presentation is associated with increased immature elements and also a high proportion with yolk sac elements (Heerema-McKenney et al. 2005). Extensively cystic tumors are usually benign, but a predominantly solid tumor raises the possibility of malignancy, though most are histologically benign if resected at delivery (Gross et al. 1987). The tumor may be so large as to obstruct labor, cause maternal injury, or rupture during delivery. The overlying skin is sometimes ulcerated and occasionally bears a rudimentary tail or digit. Some tumors are highly vascular, and telangiectasia in the overlying skin should warn of the risk of hemorrhage into the tumor or high-output heart failure. Occasionally adrenal cortical tissue may produce secondary effects as a result of secretion of steroid hormones. Sometimes the tumor is inconspicuous, marked externally only by a skin tag, dimple, or tuft of hair. Internal tumors may present with delayed passage

of meconium. Tumors may not lie in the midline and can present as a lateral mass in the buttock.

Sacrococcygeal teratoma may be entirely postsacral (external), presacral (internal), or dumbbell shaped (Sebire et al. 2004). The risk of malignancy is increased when the tumor is internal and when the diagnosis is delayed beyond the newborn period. Benign tumors are usually well defined with a solid and cystic cut surface. Some tumors are highly differentiated with well-formed bronchial or intestinal cysts. Neuroglial tissue, with or without choroid plexus, is seen in most cases and may predominate. Twenty percent to 30% of tumors contain immature tissue, usually neural and resembling neuroblastoma or the developing neural tube (Storr et al. 1997). The amount of this tissue has been graded, as in the ovary (Gonzalez-Crussi et al. 1978), but its presence does not indicate malignancy in this context (Bale 1984); rather, it should stimulate a close look for yolk sac elements, which can be very variable in appearance (Perlman 1998; Sebire et al. 2004). Immature renal tissue, striated muscle and other tissues are also seen (Fig. 15.2). Tumors with immature tissue have an increased risk of local recurrence (Sinnock et al. 1996; Perlman and Kretschmar 1997; Heerema-McKenney et al. 2005). Neural elements may recur as local implants (dermal-subcutaneous gliomatosis) without ominous significance. About 10% to 20% of sacrococcygeal teratomas presenting in the newborn period are malignant, including all those with yolk sac elements or embryonal carcinoma, and produce α-fetoprotein (Berry et al. 1969; Perlman and Kretschmar 1997; Perlman 1998; Sebire et al. 2004). Small foci of yolk sac tumor may be hard to recognize, resembling other immature epithelial structures such as intestine. It may have a variety of growth patterns, including reticular, festoon, polyvesicular, vitelline, and solid areas. Hepatoid variant of solid type may resemble fetal liver (Heifetz et al. 1998).

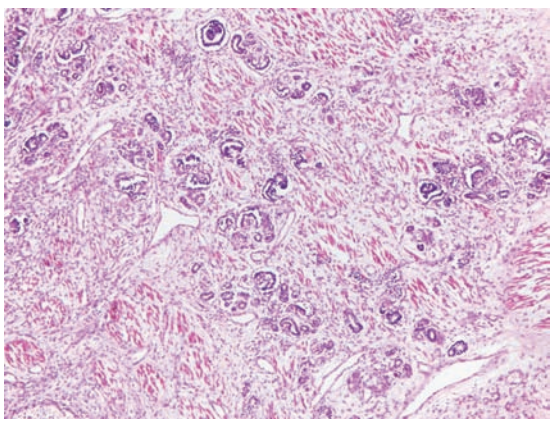

Figure 15.2. Sacrococcygeal teratoma. Term neonate with large external mass showing skeletal muscle and renal elements. Abundant neuroglial elements present in other fields.

The main objectives of pathological examination are to assess the completeness of excision and to offer a guide to prognosis. Failure to remove the coccyx results in an increased risk of recurrence, and it is important to identify and record this structure in the surgical specimen. However, failure to achieve full excision does not always lead to recurrence. The tumor must be adequately sampled, taking at least one block per centimeter diameter of the tumor and sampling areas of hemorrhage and necrosis, which may indicate malignancy. Maturation and regression of sacrococcygeal teratomas in the interval between debulking and definitive resection has been described (Graf et al. 1998). However, despite the good outcome of most immature sacrococcygeal teratomas, it is important to recognize that an occasional tumor without frankly malignant elements at presentation may recur with malignant elements and cause death (Hawkins et al. 1993). Retrospective analysis in some of these cases has revealed occult foci of yolk sac tumor in the original tumor (Hawkins et al. 1993). In other cases there may have been foci of yolk sac tumor separate from the main tumor mass (Gilcrease et al. 1995). Follow-up for at least 2 years is recommended regardless of histological type, although late recurrence may occur even in adulthood (Lahdenne et al. 1993).

Teratomas and Germ Cell Tumors at Other Sites

Other teratomas that affect the newborn include orbital, facial, intracranial (see later), cervical (especially thyroid), mediastinal, intrapericardial, and gastric teratomas. Orbital teratoma is peculiar to the newborn and has often reached a large size by the time of birth. There is exophthalmos of striking proportions. The extrinsic muscles of the eye are stretched over a retrobulbar mass, which may have an intracranial extension (Levin

et al. 1986). Differential diagnosis includes hemangioma, lymphangioma, epidermal inclusion cyst, and metastatic neuroblastoma.

Head and neck tumors have been reviewed by Lack (1985) and Carr and colleagues (1997). Teratoma involving the pharynx and base of the skull appears to arise from around Rathke's pouch. It frequently prevents fusion of the palatal processes, resulting in clefting. Large tumors project from the mouth (epignathus) and cause polyhydramnios by preventing fetal swallowing. The tumor site often precludes complete excision, and death occurs from asphyxia or from meningitis if the base of the skull is involved. Teratomas also arise in the tongue (Uchida et al. 1998).

A probably related lesion peculiar to infancy is the hairy polyp of the pharynx. This tumor-like lesion consists of a mass of fibroadipose tissue covered by hair-bearing skin projecting into the mouth or pharynx (Fig. 15.3). This entity is best regarded as a hamartoma. Meningoepithelial elements have been described (Olivares-Pakzad et al. 1995).

Cervical tumors account for 3% to 4% of childhood teratomas and are usually evident as a large mass at birth (Baumann and Nerlich 1993; Vujanic et al. 1994). Seventeen percent of affected babies are stillborn and 35% die before surgery. The EXIT procedure where the baby is delivered by cesarean section and maintained on the placenta until a surgically protected airway is established may be used if a large obstructive tumor has been identified by prenatal scans (Shih et al. 2005). Many tumors are intimately related to the thyroid, resulting in postoperative hypothyroidism and hypoparathyroidism (Riedlinger et al. 2005). Congenital cervical teratomas are usually benign, but yolk sac tumors (Dehner et al. 1990) and metastases have been described (Baumann and Nerlich 1993). Lymph nodes draining cervicothyroid teratomas may contain foci of neural tissue without ominous significance (Dehner et al. 1990; Keen et al. 1998).

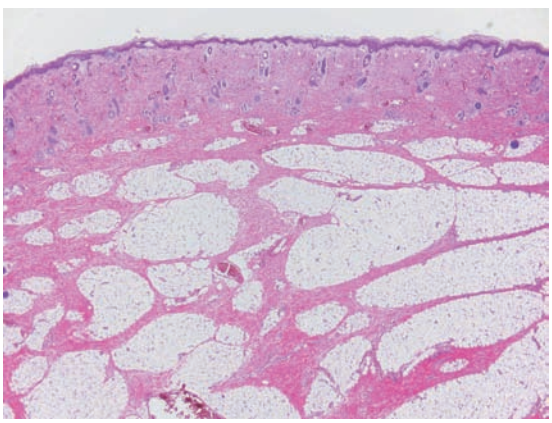

Figure 15.3. Hairy polyp. Term neonate with mass in the mouth arising from the soft palate consisting of skin-like tissue with hair and adnexal glands over fibroadipose tissue. A cartilage bar was present deep in the lesion.

Mediastinal tumors may present with hydrops fetalis or respiratory obstruction caused by compression of adjacent structures. Carter et al. (1982) emphasized the benign behavior of mediastinal teratomas in infants, even when histologically immature. Intrapericardial tumors present in infancy with cardiomegaly, muffled heart sounds, and cardiac tamponade caused by a pericardial effusion. Of 48 cases reported by Torres-Aybar et al. (1975), 24 were in infants less than 3 months old. These tumors arise close to the root of the great vessels, often on the right side, and may be fatal because of their location or, very rarely, malignant behavior.

Gastric teratomas are notable because, unlike other congenital teratomas, they occur almost exclusively in males. Malignancy is very rare (Satge et al. 1990; Bourke et al. 1997), and treatment is by local excision. Yolk sac tumor is sometimes found at extragonadal sites including the vagina, the head and neck, and the retroperitoneum. Various chromosomal abnormalities have been demonstrated and are different from those seen in yolk sac tumor in adults (Jenderny et al. 1996).

The infantile choriocarcinoma usually arises from the placenta and presents in the first few weeks with varying features including anemia, organomegaly, and sometimes precocious puberty (see later) (Blohm and Gobel 2004).

Congenital Neuroblastoma

Neuroblastoma has been diagnosed before birth by ultrasound scanning when it may rarely produce symptoms in the mother due to secretion of

catecholamines. It is the commonest malignant solid tumor presenting in the neonatal period, accounting for 30% to 50% of cases. The majority have an abdominal primary, either in the adrenal gland or retroperitoneum (Schneider et al. 1965). Pathological management is well summarized elsewhere (Qualman et al. 2005).

Rare cases are familial (Mancini et al. 1982), associated with BWS (Emery et al. 1983), or part of a disorder of neural crest such as neurofibromatosis type 1 (NF1), Hirschsprung's disease, nesidioblastosis (Grotting et al. 1979), or Ondine's curse (Stovroff et al. 1995; Rohrer et al. 2002). There also appears to be an association with congenital heart disease (George et al. 2004). There may be placental involvement with widespread plugging of villous capillaries (Lynn et al. 1997), resulting in hydrops fetalis. Concordance for neuroblastoma in twins may be due to transplacental metastasis or simultaneous onset of tumors in each twin (Boyd and Schofield 1995). Metastasis to the skin is common in neonates. Hepatic involvement, often massive, was present in 65% of the neonatal cases reviewed by Schneider et al. (1965). The terms *Pepper's syndrome* and *Hutchinson's syndrome* are no longer fashionable; the former described massive liver involvement by adrenal neuroblastoma, which is virtually confined to young infants. It has been suggested that the liver may be reseeded from the placenta via the umbilical vein, while the lungs are spared by diversion of blood through the foramen ovale and ductus arteriosus (Wieberdink 1982). Congenital neuroblastoma undergoes spontaneous regression more commonly than any other tumor. Regression is usually the result of necrobiosis, but maturation to ganglioneuroma may occur (Haas et al. 1988). This remarkable phenomenon has been well documented and accounts in part for the relatively good prognosis of neuroblastoma in young children. A cystic variant of neuroblastoma is uncommon (2% of neuroblastomas) and appears to present predominantly in neonates (Chen et al. 1997; Kozakewich et al. 1998).

The diagnosis of neuroblastoma in neonates poses the same diagnostic problems as in older children. The microscopic appearance does not differ significantly from that in older children, but its behavior and metastatic pattern are distinctive. Diagnostic aids such as the measurement of catecholamines and their metabolites in body fluids (Graham-Pole et al. 1983), immunohistochemistry, catecholamine autofluorescence, and electron microscopy may be helpful (Triche and Askin 1983). Artifactually dispersed neural tissue in macerated fetuses may be mistaken for metastatic/multifocal neural tumors (Huff 1985; Langlois and Gray 1992).

The prognosis of neuroblastoma depends on site, stage, grade, and especially age (Evans and D'Angio 2005). Pathological grading systems, which correlate with prognosis, were proposed by Beckwith and Martin (1968) and Shimada et al. (1995). Favorable prognosis correlates with low N-*myc* copy number, aneuploidy, and no loss of chromosome 1p (Joshi and Tsongalis 1997). N-*myc* amplification may be focal and localized to areas with undifferentiated morphology (Shimada et al. 1995; Lorenzana et al. 1997). A modified Shimada classification is currently preferred, with the age, stage, histology, and molecular markers taken into account for management (Joshi 2000; Shimada et al. 2001).

Various explanations have been advanced for the relatively benign behavior of neuroblastoma in neonates. About 10% of children and at least 30% of neonates with neuroblastoma have a special pattern of metastasis termed stage IV-S neuroblastoma, which has a particularly favorable outlook. It describes patients with small primary tumors and dissemination to liver, skin, and bone marrow but without bone lesions (D'Angio et al. 1971; Evans et al. 1981; Joshi 2000). Survival has been reported as 93% in babies with skin metastases compared with 32% survival when these are absent (Stephenson et al. 1986). Newborns have a poorer survival than babies presenting later with stage IV-S disease because of massive liver enlargement causing respiratory failure (van Noesel et al. 1997). Stage IV-S neuroblastoma does not usually show N-*myc* amplification, but when it does it often behaves aggressively (Bourhis et al. 1991; Shimada et al. 1995; Hachitanda and Hata 1996). Telomerase is also not usually present in stage IV-S tumors, unlike more aggressive neuroblastomas, but has been reported in a stage IV-S tumor that was fatal (Hiyama et al. 1995). Thus, it appears that most stage IV-S neuroblastomas lack the

genetic mechanisms for fully malignant behavior. Incidentally discovered connatal localized neuroblastomas may progress, and therefore some authors caution against a wait-and-see strategy (Kerbl et al. 1996), though others advocate such an approach, especially for cases detected by screening (Fritsch et al. 2004).

Small nodules of neuroblasts up to 400 µm in diameter with a modal size of 60 µm are seen in the adrenal glands of fetuses (Turkel and Itabashi 1974). They become less frequent after 20 weeks' gestation and are uncommon at birth. "In-situ neuroblastoma" refers to larger nodules of undifferentiated neuroblasts a few millimeters in diameter situated in the adrenal medulla (Beckwith and Perrin 1963). Some are visible to the naked eye (Guin et al. 1969).

The natural history of in-situ neuroblastoma is unknown. Systematic search shows in-situ neuroblastoma in 1% to 2% of routine perinatal necropsies. However, other congenital abnormalities (often cardiac) were found in 30% of these cases (Isaacs 1985), and it has been argued that it is part of a generalized dysontogenic process. If the normal newborn population has the same frequency of this lesion as in the necropsied population, then the majority of in-situ neuroblastomas must regress spontaneously, very few becoming malignant tumors. The favorable prognosis of neuroblastoma in infancy has stimulated screening programs based on catecholamine metabolites in urine to detect the tumor before it is clinically manifest. Such programs in Japan have not been successful in reducing mortality but do detect favorable prognosis neuroblastomas that would not otherwise have come to medical attention (Craft and Parker 1992). In the Quebec Neuroblastoma Screening Project, most screening positive cases were of favorable histology while most unfavorable histology tumors were missed by screening (Kawakami et al. 1998). Although the effectiveness of these programs in preventing death is still in question, it is clear that a majority of neuroblastomas are detectable by 6 months, and so in fact many may be congenital. Carlsen (1988) argues on theoretical grounds that almost all childhood neuroblastomas arise in utero. Screening for neuroblastoma does not appear to be of benefit (Tsubono and Hisamichi 2004).

Hematological Tumors

Congenital Leukemia

Congenital leukemia is rare but is the major cause of cancer deaths in the first month of life. Congenital leukemia has very different features from leukemia in older children. The prognosis is poor, though it has improved with aggressive therapy. Acute nonlymphoblastic leukemia (ANLL) is more common than acute lymphoblastic leukemia (ALL) and, once in remission, ANLL cases have a better prognosis than ALL cases (Bresters et al. 2002; Isaacs 2003). The ALL cases have a disease-free survival of under 10% in many series; 63% of patients have shown skin involvement (Bresters et al. 2002). Organomegaly is often a prominent feature, and skin and central nervous system (CNS) involvement is common (Resnik and Brod 1993; Bresters et al. 2002). Congenital leukemia may be a cause of stillbirth in up to 7% of Down syndrome fetuses and 3% of non–Down syndrome fetuses. The fetuses are usually hydropic and the placentas large and edematous (Isaacs 2003).

Leukemia must be distinguished from the florid leukemoid reactions seen in neonates. Four diagnostic criteria have been suggested: proliferation of immature cells of the myeloid or lymphoid series; infiltration of nonhemopoietic tissues; absence of other diseases that might cause diagnostic confusion, such as erythroblastosis fetalis, congenital syphilis, or viral and bacterial infections (Engle et al. 1983); and presentation before 4 weeks of age (Resnik and Brod 1993). Leukemia is associated with Down syndrome, Bloom syndrome, Turner syndrome, trisomy 13, Fanconi's anemia, Ellis–van Creveld syndrome, Schwachman syndrome, and Rubinstein–Taybi syndrome (Isaacs 2003).

Abnormalities of the mixed-lineage leukemia (MLL) gene are seen in 50% or more of congenital leukemias, in both the ANLL and ALL types (Isaacs 2003). This gene is also often involved in postchemotherapy-related leukemias. In neonates, MLL rearrangements usually are a poor prognostic marker (Sansone and Negri 1992; Isaacs 2003). Congenital leukemia is often CD10 negative, and leukemias (especially with MLL

changes) may show lymphoid and myeloid marker positivity (mixed lineage or biphenotypic leukemia). Leukemias with MLL gene translocations usually cluster together in gene profile experiments, suggesting a separate pathogenetic group (Ross et al. 2004).

A condition variously called transient abnormal myelopoiesis (TAM) or transient myeloproliferative disorder (TMD) is now often called transient leukemia (Zipursky 2003). This condition resembles the megakaryoblastic leukemia (M7), which is usually seen in trisomy 21 but commonly remits spontaneously (Fig. 15.4). The infants are generally healthy but with hepatosplenomegaly. The transient leukemia may be managed expectantly unless there is life-threatening progression. It may induce severe hepatic fibrosis as a result of the production of fibrogenic cytokines (Becroft 1993). Bone marrow tends not to show the fibrosis, perhaps because it is not the major site of hemopoiesis in fetal life. There are genetic differences in the genetic signatures of transient leukemia and acute megakaryoblastic leukemia associated with trisomy 21 (Lightfoot et al. 2004). Telomerase activity is present in the leukemia but usually not in the nonfatal transient form (Holt et al. 2002). However, a high proportion with the transient leukemia (25% or so) relapse with leukemia by the age of 4, and a number of others succumb to complications, such as infection or hematological complications (Isaacs 2003; Zipursky 2003). A similar syndrome is seen in children with trisomy 21 mosaicism, the leukemoid reaction being confined to trisomic cells with other somatic cells having a normal karyotype, and this has a relatively good prognosis (Bhatt et al. 1995). Occasionally congenital leukemia without trisomy 21 remits without treatment. Older children with Down syndrome have a 20 times increased risk of acute leukemia (Satge et al. 1998b; Hasle et al. 2000). It is thought that leukemia occurs only in those children who had transient leukemia and involves the same clone of cells. Genes present on chromosome 21 such as *AML1* and *TIAML* may have a role in this association. Transient leukemia has also been associated with Noonan syndrome (Silvio et al. 2002).

Care must be taken not to overdiagnose leukemia in chylous effusions in infancy, which can contain numerous lymphocytes and raise cytological concerns of leukemia (Horn and Penchansky 1999).

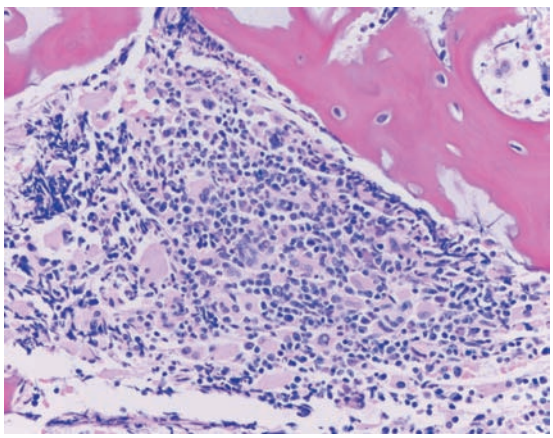

Figure 15.4. Transient leukemia. A 2-week-old with leukemic blood film. Bone marrow trephine biopsy showing excess atypical megakaryoblasts. Cytogenetics showed trisomy 21 from the blood. The infant was not dysmorphic and had a normal skin karyotype. Leukemia resolved without treatment.

Lymphoma

Lymphomas are rare in infants and almost unknown in the newborn (Tateyama et al. 1991). Congenital immunodeficiency syndromes predispose to lymphoma and leukemia in older children (Taylor et al. 1996; van den Berg et al. 1997).

Histiocytic Disorders

The biology, classification, and diagnosis of the histiocytic disorders of childhood (Favara et al. 1997) have been clarified in recent years (Weitzman and Jaffe 2005). In the newborn period, Langerhans' cell histiocytosis, hemophagocytic lymphohistiocytosis, and juvenile xanthogranuloma all pose diagnostic and prognostic problems. Rosai-Dorfman disease (sinus histiocytosis with massive lymphadenopathy, SHML) has also rarely been described in neonates (Foucar et al. 1990). Autoimmune lymphoproliferative syndrome—another disease due to defective apoptosis—usually presents later, but splenomegaly and SHML-like changes can present shortly after birth (Maric et al. 2005).

Langerhans Cell Histiocytosis

Langerhans' cell histiocytosis (LCH) presenting in neonates has been reviewed recently (Minkov et al. 2005). Multifocal disease is common and has a poor prognosis. The incidence is about 1 in 1 million neonates. About 90% of cases have skin involvement, 40% single system disease (with a 94% 5-year survival), 60% with multiple organ disease, and 75% with involvement of the liver, spleen, lung, or hematological system and have less than a 50% survival. Those presenting with multiple nodules confined to the skin in the newborn period often regress spontaneously, and this form has been called congenital self-healing reticulohistiocytosis or Hashimoto–Pritzker disease (Hashimoto et al. 1986; Longaker et al. 1994). Skin involvement with LCH also can be present as a recalcitrant eczema. Congenital LCH with systemic involvement has a less favorable outlook, but occasionally even this can regress (Broadbent et al. 1984; Minkov et al. 2005). The liver involvement may give rise to secondary sclerosing cholangitis. Gastrointestinal (GI) involvement in neonates and infants is probably underreported and can cause diarrhea, bleeding, protein-losing enteropathy, and perianal disease (Keeling and Harries 1973; Lee et al. 1990; Geissmann et al. 1996).

Studies using X-linked markers show that unifocal and multifocal LCH is clonal (Willman 1994). A definitive diagnosis of LCH requires positive immunostaining for CD1a (Emile et al. 1995) or identification of Birbeck granules by electron microscopy. Clinical behavior is determined by the extent of organ involvement and dysfunction. More aggressive disease may be indicated by age under 1 year old and multiple bone and organ involvement (Jubran et al. 2005). Occasional associations of LCH with congenital abnormalities (Sheils and Dover 1989), leukemia, and other neoplasms (Egeler et al. 1993) are recognized, and molecular similarities between the two tumors have been shown (Feldman et al. 2005).

Hemophagocytic Lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is a familial (autosomal recessive) or sporadic syndrome usually affecting infants and young children characterized by fever (>7 days), splenomegaly, cytopenias, hypertriglyceridemia, hypofibrinogenemia, and hemophagocytosis (Farquhar and Claireaux 1952; Favara 1992; Arico et al. 1996, 2001; Henter 2002; Janka and Schneider 2004).

The disease is now best classified into the following different groups, though these can be difficult to distinguish. The acquired types usually present later than 1 year of age (Janka and Schneider 2004):

- Primary genetic HLH
- Associated with other recognized immune deficiencies (e.g., Griscelli, Chediak Higashi, and XLP syndromes)
- Acquired infection-associated (viral, bacterial, fungal, or protozoal infections in previously well individuals)
- Associated with autoimmune diseases (e.g., rheumatic disease)
- Associated with malignancy
- Associated with metabolic disorder (e.g., lysinuric protein intolerance) (Duval et al. 1999)

Familial cases often have a background of consanguinity, and the disease is usually triggered by an infection (Arico et al. 1996). Histology of many organs shows an infiltrate of lymphocytes, activated non-Langerhans' histiocytes, and hemophagocytosis (Fig. 15.5). The appearance in liver biopsies has been reviewed by Favara (1996) and the postmortem findings by Ost et al. (1998). In some cases, neurological symptoms may be prominent with meningeal, perivascular, and parenchymal infiltration (Haddad et al. 1997; Henter and Nennesmo 1997). Skin involvement may be the presenting feature (Morrell et al. 2002). The disease was often missed and, as it is fatal unless treated, was diagnosed only at autopsy.

Hemophagocytic lymphohistiocytosis appears to be due to uncontrolled activation of T cells producing interleukin-2 and other cytokines due to impaired apoptosis of target cells (Henter 2002). Defective natural killer (NK) cell function has been found (Sullivan et al. 1998). Familial HLH (FLH) has been subdivided according to the genetics. FLH2 forms about 20% to 40% of FLH and is due to mutations of the perforin gene (Goransdotter Ericson et al. 2001). A second gene,

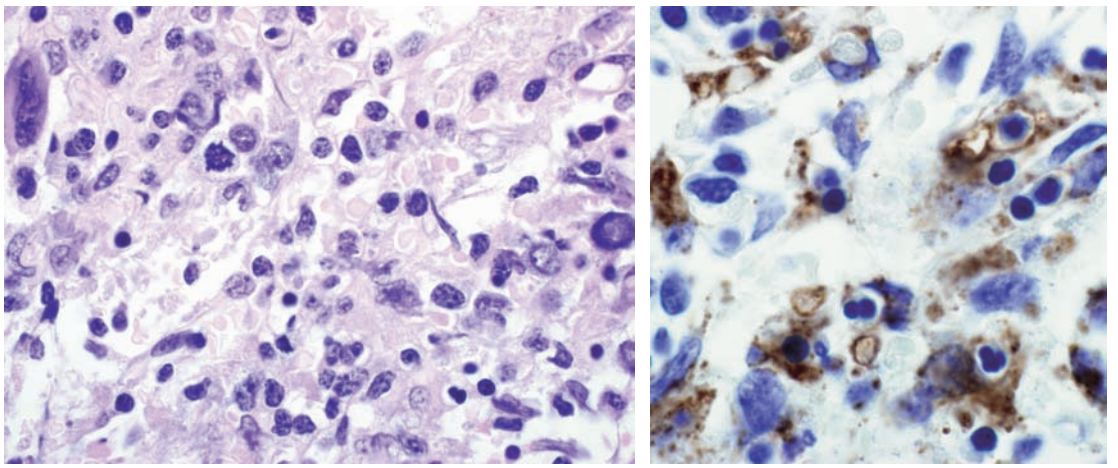

Figure 15.5. (A) Familial hemophagocytic lymphohistiocytosis. A 3-month-old with history of pallor, skin rash, hepatosplenomegaly, and pancytopenia. Bone marrow aspirate shows numerous histiocytes with hemophagocytosis. (B) Immunohistochemistry for CD68 confirms the changes. The serum triglycerides were high.

Munc 13–4 (Feldmann et al. 2003), causes FLH3, which presents later (Ishii et al. 2005).

The treatment for primary genetic HLH is chemotherapy to suppress the ineffective immune response followed by bone marrow transplantation with a 60% to 70% cure rate (Henter 2002; Janka and Schneider 2004; Horne et al. 2005).

Juvenile Xanthogranuloma

Juvenile xanthogranuloma (JXG) usually presents at birth or in infancy, usually with a single (less frequently multiple) dome-shaped nodule in the skin of the head, neck, or trunk. Biopsy shows histiocytes, foam cells, Touton giant cells, and eosinophils in varying proportions and can be classified into the early, classic, transitional, and combined subtypes (Coffin 1997). The proliferating cell is the dermal dendrocyte, which is CD68 and fascin positive; usually expresses factor Xllla, LCA, CD4, and rarely S100; and is negative for CD1a (Favara et al. 1995; Freyer et al. 1996; Kraus et al. 2001; Dehner 2003; Janssen and Harms 2005). Birbeck granules are not identified. The noncutaneous lesions tend not to have multinucleate cells, may have spindle cells, and can be difficult to diagnose (Dehner 2003). Most lesions regress spontaneously by 3 years. There is a reported association among NF1, juvenile xanthogranuloma, and juvenile chronic myeloid leukemia (Zvulunov et al. 1995; Janssen and Harms 2005).

Juvenile xanthogranuloma also occurs in a systemic form involving subcutaneous tissue, muscle, central nervous system, liver, spleen, and other sites. About 10% of babies with cutaneous JXG have extracutaneous involvement, and most, but not all, of those with multifocal disease have cutaneous lesions. Liver involvement with giant cell hepatitis can be fatal (Dehner 2003). A third of patients have lesions at birth, and it appears to be more common in males (Dehner 2003; Janssen and Harms 2005). Treatment may be difficult and the systemic form of JXG carries significant morbidity and mortality, particularly if the brain is involved (Freyer et al. 1996; Janssen and Harms 2005). Langerhans' cell histiocytosis type chemotherapy has been used for systemic disease (Nakatani et al. 2004). There may be overlap with some of the non-Langerhans histocytoses (Sidwell et al. 2005).

Congenital Soft Tissue Tumors

Mesenchymal Tumors

Mesenchymal lesions comprise about 10% of neonatal tumors. Kauffman and Stout (1965) reported 125 congenital mesenchymal tumors of soft tissue. Fibromatoses, mesenchymoma, rhabdomyosarcoma, smooth muscle tumors, and hemangiopericytoma were the commonest types in their series. Since that time, a number of new entities have been described that might necessitate reclassification of some of their cases (Coffin et al. 1997; Fletcher et al. 2002). However, their conclusion that congenital mesenchymal tumors seldom behave in a malignant way and differ biologically from those in adults is still valid (Coffin and Dehner 1990; Coffin and Dehner 1991; Spicer 1992; Dillon et al. 1995). Malignant neonatal soft tissue tumors are divided equally among congenital fibrosarcoma, rhabdomyosarcoma, and other nonrhabdomyosarcoma malignant soft tissue tumors including rhabdoid tumors (Dillon et al. 1995; Isaacs 2002b).

Vascular Tumors

The classification of hemangiomas is complicated, as cases do not always readily fit into single entities and some cases appear to be a neoplasm, others reactive, and others are a malformation. The capillary hemangioma (infantile hemangioendothelioma) is well known. It classically presents as a strawberry nevus, increasing in size for a few months before regressing. Treatment, which includes intralesional steroids and interferon, is usually reserved for those cases that affect sight or feeding or cause other significant symptom (Barrio and Drolet 2005). Diffuse involvement of the tissues—angiomatosis—is well recognized though may present later in childhood. The placenta may also be involved with diffuse hemangiomatosis (Bakaris et al. 2004). Glucose transporter 1 (GLUT1) can be used to distinguish the involutional hemangioma from the other entities (North et al. 2000; Leon-Villapalos et al. 2005). Two new infantile capillary hemangioma variants, the rapidly involutional capillary hemangioma (RICH) and the noninvoluting capillary hemangioma (NICH) variant, have been recently described (Berenguer et al. 2003). They have histological appearances that overlap the common infantile hemangioma, although they are GLUT1 negative.

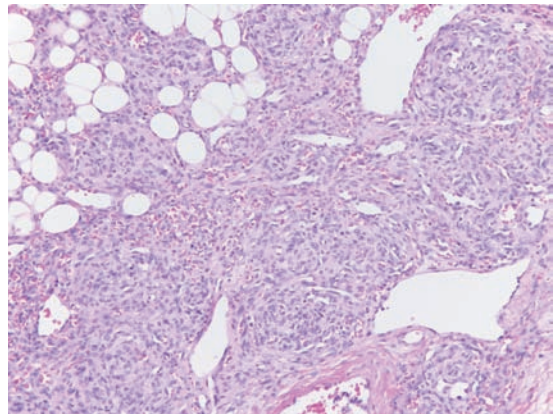

Figure 15.6. Kaposiform hemangioendothelioma. A 3-month-old with soft tissue mass increasing in size since birth, who also developed Kasabach-Merritt syndrome. Adipose tissue infiltrated by vascular nodules with spindle cells and with larger vessels adjacent to the nodules.

The tufted hemangioma often appears more plaque-like and generally develops after birth (Wong and Tay 2002). Kaposiform hemangioendothelioma is a tumor often presenting in infancy and increasingly recognized in adults (Fig. 15.6). It may present at birth as a mass (Lyons et al. 2004) or a cause of hydrops (Martinez et al. 2004). It does not fully regress. It may closely resemble infantile hemangiomas, especially in small biopsies, but it is negative for GLUT1, and the lymphatic marker D2–40 stains the spindle cell and lymphatic components (Debelenko et al. 2005).

The Kasabach-Merritt syndrome is associated with the kaposiform hemangioendothelioma and the tufted angioma, but also may be seen in other entities (Enjolras et al. 1997; Alvarez-Mendoza et al. 2000; Hall 2001). Rarely, it appears to be the cause of hypothyroidism (Guven et al. 2005). There are several syndromes associated with hemangiomas including the PHACE syndrome (Metry et al. 2001), (Posterior fossa malformations, Hemangiomas, Arterial anomalies, Coarctation of aorta and other cardiac defects, and Eye abnormalities). Another

Table 15.7. Fibrous proliferations of infancy and childhood

Congenital myofibromatosis
Solitary
Multicentric
Infantile desmoid-type fibromatosis
Gardner-associated fibroma
Fibromatosis colli (sternomastoid tumor)
Infantile digital fibromatosis (recurring digital fibroma)
Cranial fasciitis
Inflammatory myofibroblastic tumor
Infantile fibrosarcoma
Giant cell fibroblastoma/dermatofibrosarcoma protuberans
Fibrous hamartoma of infancy
Calcifying aponeurotic fibroma
Plantar fibromatosis
Juvenile hyaline fibromatosis
Gingival fibromatosis
Fibrodysplasia (myositis) ossificans progressiva
Plexiform fibrohistiocytic tumor
Fibrolipomatosis
Gastrointestinal stromal tumor (see above)

entity in infants is the giant cell angioblastoma (Vargas et al. 2001).

Lymphangiomas are common tumors in neonates. They are often diffuse, poorly localized, and difficult to remove, and they often recur. A new immunohistochemical marker D2–40 that appears to distinguish lymphatic from vascular endothelium has been described (Galambos and Nodit 2005).

Fibromatoses

The fibrous proliferations of infancy and childhood include a number of distinctive lesions unique to this age group that are recognizable as much by their clinical presentation as by their histology (Table 15.7) (Coffin and Dehner 1991; Coffin et al. 1997; Fisher 1996). All have in common the proliferation of spindle cells resembling fibroblasts, which are locally invasive, but generally lack the capacity to metastasize. Fibromatoses seen in infancy are usually more cellular than adult types, although the latter also occur in childhood. The discussion here focuses on the fibrous proliferations that are seen in neonates and young infants. The infantile fibromatosis, myofibromatosis, hemangiopericytoma, and congenital fibrosarcoma tumors often show considerable histological overlap, and an individual lesion may show features of more than one entity (Variend et al. 1995). Most authorities now regard hemangiopericytoma of infancy as a myofibromatosis (Fisher 1996).

Congenital Myofibromatosis (Solitary and Multicentric)

This distinctive lesion of neonates has been described under a variety of synonyms (Chung and Enzinger 1981; Wiswell et al. 1988; Coffin and Dehner 1991). Congenital myofibromatosis may be solitary, usually involving the soft tissues of the head, neck, and trunk, and is more common in males. Multicentric involvement of the skin, soft tissue, and bone is less common but usually has a good outcome with spontaneous regression. The third and rarest type, generalized myofibromatosis with visceral organ involvement, is often fatal (Coffin et al. 1995). Sixty percent of lesions are congenital. Autosomal dominant (with variable penetrance) and possibly recessive modes of inheritance have been suggested in some cases (OMIM 228550) (Jennings et al. 1984; Zand et al. 2004). Various chromosomal changes have been reported in the tumors.

Myofibromatoses are more or less well demarcated, often vascular nodules up to several centimeters in diameter. Occasional examples are cystic. Microscopically, they tend to be cellular and are composed of nodules of plump spindle cells showing mitotic activity. The center of the lesion characteristically has a hemangiopericytomatous pattern, while the periphery of the tumor appears mature with more collagen. Giant cells may rarely be present. Necrosis, intravascular growth, trapped fat, and an infiltrative margin may be present but are not of adverse significance. Congenital myofibromatoses are immunohistochemically positive for smooth muscle actin, vimentin, and desmin (Fletcher et al. 1987; Variend et al. 1995; Magid et al. 1997), and the histogenesis is believed to be from myofibroblasts. Coffin et al. (1995) suggested that the generalized type arises multifocally from subintimal mesenchymal or smooth muscle cells with a myofibroblastic phenotype. One case has been associated with fibromuscular dysplasia of the arteries (Wright et al. 2004).

Although solitary lesions sometimes recur, they do not metastasize, and the outlook is excellent.

The prognosis is less good for patients with multicentric visceral lesions due to involvement of vital organs, particularly the lungs, where there may be occlusion of pulmonary veins (Coffin et al. 1995). New lesions may continue to appear after birth. Spontaneous regression, which may be by apoptosis, can occur even in patients with multiple lesions (Fukasawa et al. 1994).

Infantile Desmoid-Type Fibromatosis

Infantile desmoid-type fibromatosis is the childhood equivalent of the desmoid tumor (musculoaponeurotic fibroma) of adults (Dormans et al. 2001). It arises in muscle, aponeurosis, or fascia. Muscles of the head and neck, shoulder, upper arm, and thigh are favored sites. The macroscopic appearance is of an ill-defined mass of gray–white tissue that infiltrates surrounding tissues at the periphery. The histological appearance varies. Some resemble the adult desmoid tumor, but more cellular examples are indistinguishable from infantile fibrosarcoma. Another pattern seen in very young infants is of immature cells, intermediate in form between primitive mesenchymal cells and fibroblasts, scattered in a myxoid background and surrounded by abundant reticulin. This appearance may be confused with other myxoid tumors such as a lipoblastoma or rhabdomyosarcoma. This fibromatosis characteristically infiltrates muscle when it may be accompanied by adipocytes.

Infantile desmoid-type fibromatosis tends to recur and infiltrate locally but does not metastasize. It may spontaneously regress. Local recurrence is correlated with the presence of numerous slit-like blood vessels and undifferentiated mesenchymal cells (Schmidt et al. 1991). Local excision is the treatment of choice but may be difficult because of the involvement of vital structures. Chemotherapy or hormone therapy have also been tried, and the tumors may express hormone receptors (Buitendijk et al. 2005). Some patients may have congenital abnormalities (Ayala et al. 1986), and 15% are associated with familial polyposis/Gardner syndrome (Dormans et al. 2001). This association should be particularly considered with less dense fibromas with features of nuchal type fibroma or with other features of this syndrome (Wehrli et al. 2001).

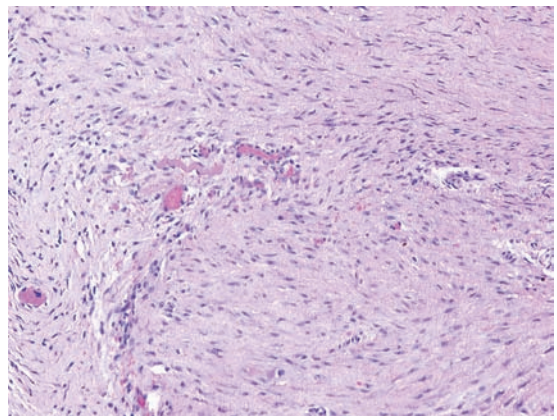

Figure 15.7. Sternomastoid tumor. A 3-week-old with mass lying arising from the anterior aspect of the sternomastoid muscle. Entrapped skeletal muscle present.

Fibromatosis Colli

Fibromatosis colli (sternomastoid tumor) typically occurs as a horizontally mobile mass in the sternomastoid muscle of an infant presenting between the second and fourth week of life (Cheng et al. 1999). After a period of growth it becomes static and may regress, to be followed by shortening of the sternomastoid muscle resulting in torticollis. In over 50% of cases there is a history of complicated delivery or trauma, and some cases also have congenital dislocation of the hip. Occasional cases occur in families (Campbell and Pedra 1956; Macdonald 1969). Microscopy shows proliferating fibroblasts surrounding regenerating residual striated muscle cells, which mimics fibromatosis, but the location and the history are important diagnostic clues (Fig. 15.7). This lesion never behaves aggressively (Coffin and Dehner 1991) and may well be a reaction to trauma to the sternomastoid. Treatment is by passive stretching or division of the sternomastoid muscle (Cheng et al. 1999).

Infantile Digital Fibromatosis

Another lesion unique to young children is the infantile digital fibromatosis (Reye's recurring digital fibroma). It appears as a dome-shaped swelling on the sides or dorsum of the middle and distal interphalangeal joints of the fingers and toes (usually sparing the great toe and

thumb). More than one lesion may be present. Most patients are less than 1 year of age and the lesion is congenital in one third of cases. Microscopic examination shows a uniform proliferation of fibroblasts and myofibroblasts (Yun 1988). A characteristic feature is the presence of a variable number of eosinophilic cytoplasmic inclusions with filamentous ultrastructure staining immunohistochemically for actin (Mukai et al. 1992). A histologically identical lesion has been described in an extradigital location in a 2.5-year-old child (Purdy and Colby 1984) and occasionally in adults (Viale et al. 1988; Pettinato et al. 1991).

The lesions tend to regress (Azam and Nicholas 1995; Kawaguchi et al. 1998), and surgery is required only for diagnosis or if function is affected. As the parenthetical term above implies, there is a tendency to local recurrence after excision (Azam and Nicholas 1995). They may be syndromic with limb eye and skin malformations (Breuning et al. 2000), and some may not have inclusions (Horii et al. 1998).

Cranial Fasciitis

Cranial fasciitis affects infants and young children and may be congenital (Lauer and Enzinger 1980). It presents as a rapidly growing mass in the soft tissue of the scalp, often eroding the outer and even inner table of the skull. Histologically, it is composed of a proliferation of loosely arranged fibroblast-like cells and shares features with nodular fasciitis (Keyserling et al. 2003). Other fasciitis-like lesions can occur in extracranial sites in young children, mainly in the head and neck, and like cranial fasciitis tend to have a more consistently uniform myxoid appearance than nodular fasciitis (Sarangarajan and Dehner 1999).

Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumor is occasionally seen in infants with the same histology as children and adults. Younger children often have abnormalities involving the *ALK* gene at 2p23, and the increased expression of this gene can be detected immunohistochemically (Coffin et al. 2001; Cook et al. 2001). Sebire et al. (2002) have described intravascular tumors.

Giant Cell Fibroblastoma/Dermatofibrosarcoma Protruberans (DFSP)

Giant cell fibroblastoma is an uncommon benign tumor of the subcutaneous tissue and dermis that has rarely been reported in the newborn (Fletcher 1988), most cases being diagnosed by 10 years of age. The histological appearance is distinctive, with solid areas composed of bland spindle cells, and "angiectoid" areas with irregular branching spaces lined by mononuclear cells and bizarre giant cells containing pale amorphous material staining as acid mucopolysaccharide. This lesion has infiltrative margins and frequently recurs after surgery. This lesion has been described with dermatofibrosarcoma protruberans (Harvell et al. 1998), which has also been described in neonates (Terrier-Lacombe et al. 2003; Gu et al. 2005). They share a specific translocation, t(17;22), involving the *COL1A1-PDGFB* gene fusion (Pedeutour et al. 1996; Simon et al. 1997). Giant cell fibroblastoma and dermatofibrosarcoma protruberans are closely related clinically, histologically, and genetically (Terrier-Lacombe et al. 2003).

Congenital (Infantile) Fibrosarcoma

Congenital fibrosarcoma is a different entity from adult-type fibrosarcoma and has a better prognosis. About half the cases of infantile fibrosarcoma described by Chung and Enzinger (1976) were present at birth and occurred preferentially in the extremities. Some lesions are very large and grow rapidly. The tumor is poorly circumscribed and tends to infiltrate adjacent tissues. The microscopic appearance may vary from that of an adult-type fibrosarcoma to a tumor composed of immature spindle cells with little evidence of fibroblastic differentiation. An infiltrate of chronic inflammatory cells is commonly seen. There may be hemangiopericytomatous areas and foci of necrosis (Coffin et al. 1994). Congenital fibrosarcoma characteristically shows trisomy of chromosome 11 (Bernstein et al. 1994). A specific translocation, t(2;15) (p13;q25), has been identified in the infantile fibrosarcoma (Knezevich et al. 1998a,b; Rubin et al. 1998) and is also found in cellular mesoblastic nephroma but not in adult-type fibrosarcoma, myofibromatosis,

or infantile hemangiopericytoma (Bourgeois et al. 2000).

Metastases occur in less than 10% of cases and the 5-year survival rate is 84% (Neifeld et al. 1978; Nonaka and Sun 2004). Two fatal cases are reported in which the recurrence showed transition to a malignant fibrous histiocytoma-like appearance (Salloum et al. 1990). The recommended treatment is wide local excision (Cofer et al. 1996) or combined with chemotherapy (Loh et al. 2002), especially for those with the translocation (McCahon et al. 2003). Exceptional cases do not recur after incomplete excision or regress spontaneously (Wilson et al. 1990; Madden et al. 1992; Miura et al. 2002). The distinction between congenital fibrosarcoma and other cellular spindle cell lesions of infancy may sometimes be difficult or impossible by microscopy alone, and infantile fibrosarcoma has been overdiagnosed in the past (Trobs et al. 1999).

Fibrous Hamartoma of Infancy

Fibrous hamartoma of infancy occurs in the lower dermis and subcutaneous tissue as a rapidly growing solitary soft mass that rarely involves the superficial aspect of underlying muscle. About 20% of cases are congenital (Dickey and Sotelo-Avila 1999). Favored sites are around the shoulder and upper arm, particularly the axillary folds, thigh, and inguinal region. Grossly it appears as an ill-defined fibrofatty lump. Microscopically it is made up of variable amounts of three components: trabeculae of fibrous tissue, mature fat, and areas of immature mesenchymal cells often arranged in nests. Sotelo-Avila and Bale (1994) emphasized the presence of thick, wide capillaries and lymphocytes in these immature areas and discuss the differential diagnosis of equivocal cases. This lesion is benign but may rarely recur locally (Dickey and Sotelo-Avila 1999). The natural history of untreated lesions seems to be rapid growth until 5 years, after which growth slows (Efem and Ekpo 1993). It is unclear whether it is a true hamartoma or a benign neoplasm, though a t(2;3) translocation has been found (Lakshminarayanan et al. 2005). The differential diagnosis of fibrous hamartoma of infancy includes lipoblastoma and three recently described entities. These are the plexiform fibrohistiocytic tumor (Enzinger and Zhang 1988; Remstein et al. 1999; Leclerc et al. 2005), lipofibromatosis (Fetsch et al. 2000), and the pre-calcaneal congenital lipofibromatosis hamartoma (Ortega-Monzo et al. 2000). The last may be familial.

Hyalinosis and Juvenile Hyaline Fibromatosis

Two overlapping autosomal recessive syndromes are described in the literature: infantile systemic hyalinosis (Landing and Nadorra 1986; Glover et al. 1991) and juvenile hyaline fibromatosis (Senzaki et al. 1998; Urbina et al. 2004). They appear due to a mutation in the capillary morphogenesis protein 2 (Dowling et al. 2003; Hanks et al. 2003). They are characterized by multiple dermal and subcutaneous nodules with onset in early infancy. Other features include gingival hypertrophy, flexion contractures, muscle weakness, intraosseous lesions, and tumorous involvement of internal organs, with the systemic type presenting earlier and with more severe features. The lesions consist of fibroblasts and thick collagen bundles widely separated by eosinophilic hyaline material due to the disrupted cell to matrix interaction and basement membrane formation.

Others

A rare entity sometimes associated with other syndromic changes is gingival fibromatosis (Tay et al. 2001). Fibrodysplasia myositis ossificans progressiva can present very early in infancy. The soft tissue swellings may be misdiagnosed if the characteristic skeletal changes have not been identified by the referring clinician (Smith 1998).

Extrarenal Rhabdoid Tumor

The rhabdoid tumor group includes the renal and liver rhabdoid tumor, the atypical teratoid/rhabdoid tumor of the brain, and the soft tissue extrarenal rhabdoid tumor in infants. Extrarenal rhabdoid tumor has been a less well defined entity than its renal counterpart. Congenital disseminated malignant rhabdoid tumor has been

described as a distinct entity with abnormalities of 22q11, placental involvement, and rapid progression to death (White et al. 1999). Soft tissue rhabdoid tumors in the skin are recognized (Hsueh and Kuo 1998) as well as other sites (Pizer et al. 1997). This group of tumors is associated with *INI* gene mutations (Biegel et al. 2000) and has a poor prognosis. In older children and adults the rhabdoid phenotype is less specific and often proves to be a result of dedifferentiation of many other tumor types (Parham et al. 1994; Wick et al. 1995).

Rhabdomyosarcoma

Of 3217 eligible patients evaluated in the Intergroup Rhabdomyosarcoma Study, 14 were less than 30 days old at diagnosis (Lobe et al. 1994). Half the tumors were caudal (buttock, sacrococcygeal, perirectal, urogenital) and the rest involved different sites. Rhabdomyosarcoma may arise in the eyelid in the newborn (Ellenbogen and Lasky 1975; Harlow et al. 1979). Most rhabdomyosarcomas in babies are embryonal and behave as in older children (Lobe et al. 1994). Alveolar rhabdomyosarcoma, which may rarely present in early life, can present with skin nodules and is a highly malignant tumor (Kitagawa et al. 1989; Dillon et al. 1995; Ito et al. 1997; Brecher et al. 2003). In older children, it usually has characteristic translocations involving the *PAX* genes (Davis and Barr 1997). The type of translocation appears to correlate with the site, age, and prognosis, with the t(1;13) being more peripheral and occurring in younger patients with a better prognosis (Sorensen et al. 2002). However, congenital alveolar rhabdomyosarcoma appears not to have these translocations despite similar histological appearances (Grundy et al. 2001).

It is important to distinguish rhabdomyosarcoma from fetal rhabdomyoma, which resembles fetal muscle and is benign (Di Sant'Agnese and Knowles 1980). Fetal rhabdomyoma is sometimes associated with Gorlin's nevoid basal cell carcinoma syndrome (DiSanto et al. 1992).

Neural Tumors

Plexiform neurofibromas are usually congenital but may appear in the first year of life and are almost pathognomonic of NF1. Other stigmata such as multiple café-au-lait spots and freckling of the axillary and inguinal skin folds are highly suggestive of NF1 but may be absent in young babies (Matsui et al. 1993; Gutmann et al. 1997). Café-au-lait spots are also seen in Noonan syndrome and Watson syndrome. Other tumors developing in children with NF1 include congenital peripheral neuroectodermal tumor (PNET), malignant schwannoma, optic nerve glioma, astrocytoma, rhabdomyosarcoma, and leukemia (Yang et al. 1995; Gutmann et al. 1997).The *NF1* gene is a tumor suppressor gene on chromosome 17 encoding neurofibromin. Neurofibromatosis type 2 (NF2) is rare and usually manifests in later childhood or adult life, often with bilateral acoustic neuromas. The gene responsible is on chromosome 22 and codes for schwannomin. Occasional patients present with segmental NF1 or NF2 affecting only part of the body as a result of somatic mosaicism (Gutmann et al. 1997; Tinschert et al. 2000).

Congenital malignant peripheral nerve sheath tumors are vanishingly rare. The congenital and childhood plexiform (multinodular) cellular schwannoma should be considered in this age group. It can have a concerning histological appearance (Woodruff et al. 2003).

Peripheral primitive neuroectodermal tumor is rare in neonates though occasionally reported (Lee et al. 2000). Benign triton tumor/neuromuscular hamartoma may present as a congenital tumor, usually in the trunk, and is composed of bundles of skeletal muscle fibers and nerves (Mitchell et al. 1995).

Melanotic Neuroectodermal Tumor of Infancy (Retinal Anlage Tumor)

Another tumor of neuroectodermal origin virtually confined to infancy is the melanotic progonoma, or melanotic neuroectodermal tumor of infancy. It has features suggesting a relationship to retinal development. Over 90% occur in the head and neck region and characteristically involve the maxilla (Pettinato et al. 1991). Other sites include the epididymis (Calabrese et al. 1995), mediastinum, thigh, and brain (Rickert et al. 1998). The histological appearance is striking. It is a biphasic tumor composed of nests of

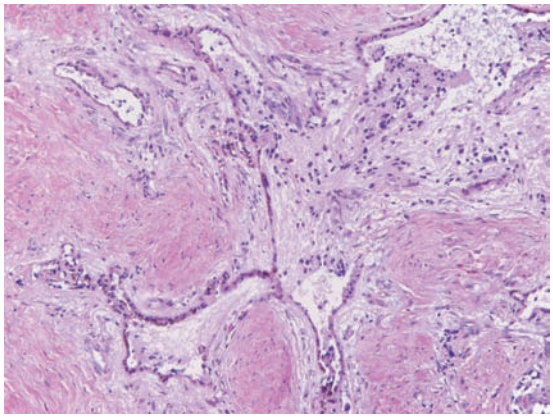

Figure 15.8. Melanotic neuroectodermal tumour. Mass started to become prominent around ear from 6 weeks of age.

neuroblast-like cells and groups of larger cuboidal cells containing melanin, sometimes forming gland-like structures, set in a connective tissue stroma (Fig. 15.8). Although the local recurrence rate is 15%, metastasis is rare, and most cases follow a benign course (Johnson et al. 1983; Carpenter et al. 1985). The small neuroblast-like cells may stain for vimentin and neuron-specific enolase, the larger cells for HMB 45 (Calabrese et al. 1995; Nelson et al. 1995; Barrett et al. 2002). Increased mitotic activity may correlate with behavior (Barrett et al. 2002). Chemotherapy may be of benefit in some cases (Woessmann et al. 2003).

Adipose Tumors

Lipoblastoma is a tumor of early childhood, occasionally present at birth, composed of lobules of immature adipose tissue containing lipoblasts with a prominent capillary network and myxoid foci reminiscent of adult myxoid liposarcoma (Mentzel et al. 1993). Liposarcomas are virtually unknown in infants, and do not show the lobulation characteristic of lipoblastoma. Lipoblastoma may mature to resemble lipoma in sequential biopsies (Collins and Chatten 1997), and may be considered an embryonal tumor of soft tissue recapitulating organogenesis (Coffin 1994). Diffuse lesions termed lipoblastomatosis have a less lobular architecture and commonly infiltrate muscle and involve deep structures. Both lesions are benign, but the diffuse form has a tendency to recur locally. Lipoblastoma shows a consistent rearrangement of chromosome 8 distinct from the rearrangement described at 12q14 in childhood and adult lipomas (Fletcher et al. 1993).

Lipomatosis of the nerve (neural lipofibroma) may be associated with macrodactyly (Murphey et al. 2004). Multiple congenital lipomatous hamartomas occur in Haberland syndrome (Rubegni et al. 2003).

Chest Wall Hamartoma

This expansile extrapleural lesion of the chest wall involving one or more ribs most often presents in newborns. It consists of nodules of proliferating hyaline cartilage, immature mesenchyme, reactive bone, and prominent vessels with hemorrhage, giving an aneurysmal bone cyst-like appearance (Psaila et al. 1996; Troum et al. 1996). A similar lesion has been described in the upper respiratory tract of newborns and young infants (McDermott et al. 1998).

Renal Tumors

Between 5% and 7% of congenital tumors arise in the kidney. Some may be detected by antenatal ultrasound (Ritchey et al. 1995). Although in the past most were called Wilms' tumors, it is now recognized that nephroblastoma is extremely rare in neonates, and that other renal tumors predominate in this age group. Pathological management is well summarized elsewhere (Qualman et al. 2003).

Congenital Mesoblastic Nephroma

Congenital mesoblastic nephroma is the commonest neonatal renal tumor (Bolande 1976). It is unrelated to Wilms' tumor and usually does not have the same associations, although occasional cases have been described with nephrogenic rests (Marsden and Newton 1986; Vujanic et al. 1995) and one with BWS (Sutherland et al. 1997). Pregnancy may be complicated by polyhydramnios or fetal hydrops (Fung et al. 1995). Mesoblastic nephroma may cause hypertension or hypercalcemia in the affected baby, which resolves rapidly

on removal of the tumor (Chan et al. 1987). The tumor is usually found on routine examination in the neonatal period, and presentation is unusual beyond 3 to 6 months of age.

The kidney is enormously enlarged by an intrarenal mass whose cut surface resembles a uterine fibroid, although the cellular variant can be extremely soft and friable. About 25% extend beyond the kidney, and nephrectomy with complete removal of the tumor is usually adequate therapy though chemotherapy may reduce the size. Histology shows a spindle cell tumor composed of bland myofibroblasts infiltrating normal kidney at its margins (Fig. 15.9). Entrapped renal tubules and glomeruli may show nuclear atypia, which is without adverse significance. Renin can be demonstrated by immunohistochemistry in these entrapped renal elements (Cook et al. 1988). Included renal tissue may also explain why this tumor takes up technetium 99 m (Tc-99 m) dimethylsulfonic acid (DMSA) and excretes contrast medium (Cowling et al. 1993). Negative immunohistochemistry for WT1 and Bcl-2 may help distinguish mesoblastic nephroma from stromal Wilms' tumor, which is positive for both (Shao et al. 2004). It must also be distinguished from metanephric stromal tumor (see below). Marsden and Newton (1986) drew attention to foci of cartilage, squamous islands, large multinucleated cells, and adrenal cytomegaly in occasional cases.

Mesoblastic nephromas with increased cellularity and mitotic activity are called cellular variants or atypical mesoblastic nephroma (Joshi et al. 1986; Marsden and Newton 1986). The latter term is a misnomer because the cellular variant is more common than the so-called classic type, particularly in older infants. Mixed classic and cellular tumors are not uncommon, suggesting the possibility of a clonal progression from classical to cellular mesoblastic nephroma (Kovacs et al. 1987; Mascarello et al. 1994). The cellular or atypical tumors may be aneuploid and show both trisomy 11 and the translocation t(12;15) (Schofield et al. 1993; Mascarello et al. 1994; Dal Cin et al. 1998; Knezevich et al. 1998; Rubin et al. 1998). The translocation, which results in *ETV6–NTRK3* gene fusions, and the trisomy 11 are also seen in infantile fibrosarcoma, and these two tumors are now considered to be closely related or the same. Both genetic changes are needed for transformation (Morrison et al. 2002). Although both infantile fibrosarcoma and cellular mesoblastic nephroma generally behave benignly, there are occasional reports of local recurrence of congenital mesoblastic nephroma and metastasis to lung and brain (Yazaki et al. 1982; Steinfeld et al. 1984; Heidelberger et al. 1993; Vujanic et al. 1993; Schlesinger et al. 1995). In the study of Gormley et al. (1989) the finding of positive surgical margins was the only significant variable predicting recurrent disease while all infants with cellular mesoblastic nephroma and clear pathological margins were cured by surgery alone. A conservative approach is therefore generally advocated, reserving chemotherapy for those few cases that recur or are not amenable to resection (Beckwith and Weeks 1986; Barrantes et al. 1991; McCahon et al. 2003).

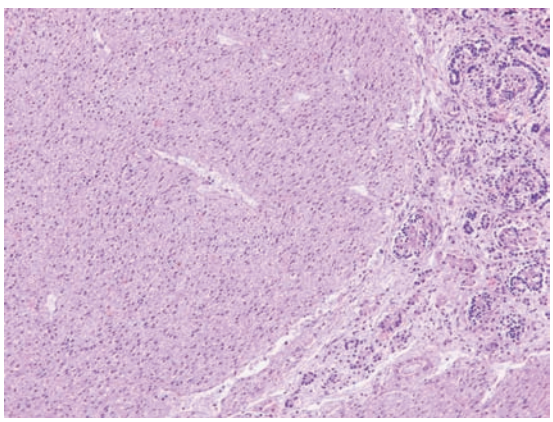

Figure 15.9. Mesoblastic nephroma. Mildly macerated stillbirth with renal mass identified at autopsy.

Metanephric Tumors

A review of the U.S. National Wilms' Tumor Study series led to the recognition that a significant number of the tumors originally diagnosed as mesoblastic nephromas were actually metanephric stromal tumors. The main histological difference between the two is in the nodular cellularity, onion skin–like cuffs around entrapped tubules, and the characteristic vascular changes, which have been associated with surgical complications (Argani and Beckwith 2000). The metanephric adenofibroma and the metanephric adenoma are

the other members of the metanephric group of tumors, which tend to occur in children and young adults and not in neonates (Arroyo et al. 2001). The metanephric adenoma needs to be distinguished from Wilms' tumor, nephrogenic rests, and embryonal hyperplasia of Bowman's epithelium (Fischer et al. 2004).

Nephroblastoma (Wilms' Tumor)

Of the 30 renal tumors in the first year of life seen by Marsden and Lawler (1983), 15 were classic Wilms' tumors, and one of these might be considered congenital. The U.S. National Wilms' Tumor Study 1969–1993 found only 0.16% of nephroblastomas occurred in the first month of life (Ritchey et al. 1995). A high proportion (64%) of these babies had associated nephrogenic rests. Gordon et al. (1996) described synchronous bilateral Wilms' tumor in a neonate. Special histological types such as the fetal rhabdomyomatous nephroblastoma (in which the major component is differentiating striated muscle), pure epithelial Wilms' tumor, and cystic partially differentiated nephroblastoma are more common in the first year of life (Joshi 1979; Ugarte et al. 1981). Wilms' tumor with anaplasia is rare under a year of age. Nephroblastoma in the first month of life is usually of low stage and has an excellent prognosis with current therapy (Ritchey et al. 1995).

Nephroblastomatosis and Nephrogenic Rests

Nephroblastomatosis has been defined as the persistence of metanephric blastema beyond 36 weeks' gestation when nephrogenesis ceases, or in an inappropriate location or amount before that time (Bove and McAdams 1976; Machin 1980; Beckwith et al. 1990). It is of great interest as a putative precursor of Wilms' tumor, being present in less than 1% of neonatal necropsies but in 20% to 40% of kidneys with Wilms' tumor. In the neonatal period rests present in two ways, either as a rare cause of bilateral renal enlargement, or as an incidental macroscopic or microscopic postmortem finding, when it should alert the pathologist to the possibility of associated syndromes. It has been described after in utero aspirin intoxication (Bove et al. 1979). In older children it is seen in kidneys removed for Wilms' tumor and occasionally in association with cystic renal dysplasia (Craver et al. 1986; Dimmick et al. 1989; Beckwith 1992) (Fig. 15.10).

Commonly, nephroblastomatosis involves the periphery of the renal lobe in a subcapsular position or along the columns of Bertin. Microscopic and discrete nests of embryonal renal epithelial cells with minor tubular differentiation and no embryonal stromal component situated beneath the renal capsule, sometimes with immature glomeruli, are termed perilobar rests. Bove and MacAdams (1976) described differentiation or maturation into other types with the formation of tubules or sclerotic changes. Hyperplastic lesions arise within rests with progression from adenoma to incipient Wilms' tumor. Beckwith et al. (1990) proposed a new and unifying classification using the term *nephrogenic rest* for a single focus of persistent nephrogenic cells and restricting the use of nephroblastomatosis to cases with multiple or diffuse nephrogenic rests. Nephrogenic rests are divided according to their location (intralobar or perilobar) and subclassified by gross and microscopic appearance (dormant, maturing, hyperplastic, or neoplastic). Nephroblastomatosis may be perilobar, intralobar, combined, or universal. This authoritative scheme is comprehensible, biologically relevant, and clinically meaningful, and so has been generally adopted.

Perilobar rests are seen in patients with BWS, hemihypertrophy, pseudohermaphrodit-

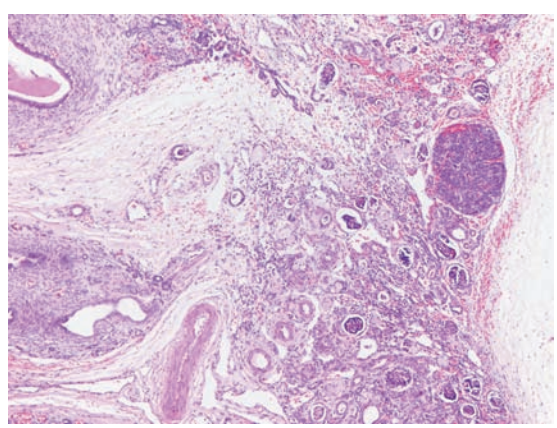

Figure 15.10. Perilobar nephrogenic rest associated with renal dysplasia. Small infant with removal of nonfunctional kidney.

ism, trisomy 18 and 13 (Satge and Van Den Berghe 1996), and some familial cases of Wilms' tumor. The nephroblastomas that develop in this form of nephroblastomosis tend to show epithelial or blastemal predominance, with scanty stroma and rare heterotopic tissues such as striated muscle. Rarely, persistent blastema forms a continuous layer around each renal lobe, resulting in considerable renal enlargement (Regalado et al. 1994). There may be tubule formation but no stromal elements. Machin (1980) reviewed nine cases, several of which presented in early infancy. Perilobar type rests are also seen associated with renal dysplasia (Craver et al. 1986).

Intralobar nephrogenic rests involving the juxtamedullary cortex are associated with germline *WT1* mutations (WAGR and Denys–Drash syndrome) and development of nephroblastoma with a predominant striated muscle component (Kikuchi et al. 1992). These rests have a higher malignant potential than perilobar rests. These rests are found in the lobule, sometimes in the medulla, and tend to be less well circumscribed and blend with the adjacent kidney. Both the cortex and the medulla may be diffusely affected, resulting in marked nephromegaly (Hou and Holman 1961; Machin 1980; Regalado et al. 1994).

The microscopic appearance of nephrogenic rests and nephroblastoma is often indistinguishable in a biopsy, and so the macroscopic appearance of the lesion is critical (Beckwith et al. 1990). The precise risk of developing Wilms' tumor in each type of rest is unknown but most nephrogenic rests probably do not become neoplastic, particularly those found incidentally (usually perilobar rests). Nephroblastomatosis in the presence of Wilms' tumor is usually bilateral and indicates the possibility of bilateral nephroblastoma, so renal tissue should be conserved whenever possible. Nephroblastomatosis is not malignant, and it is reasonable to follow most patients by regular imaging of both kidneys. If there is progression, then chemotherapy is effective and probably reduces the malignant potential of nephroblastomatosis (Beckwith 1993). Occasional cases of unilateral nephroblastomatosis can be explained by somatic mosaicism (Chao et al. 1993). Of note, if an infant is found with nephrogenic rests, the risk of a metachronous Wilms' tumor is very high (especially with perilobar rests) (Coppes and Beckwith 2000). The management of these cases is not easy and the large cancer groups, such as the Children's Oncology Group, are implementing protocols for infants and children with rests and Wilms' tumor syndromes.

Juxtarenal and extrarenal Wilms' tumors are believed to arise from heterotopic nephrogenic tissue (Wakely et al. 1989). Renal elements resembling nephrogenic rests or cystic renal dysplasia are occasionally seen in the sacral region in association with spinal dysraphism (Cozzutto et al. 1983; Alston et al. 1989), and extrarenal Wilms' tumor has been reported at this site in a patient with spina bifida (Mirkin et al. 1990) (Fig. 15.2). Mesoblastic tissue can also occasionally be seen adjacent to the gonads at fetal autopsy.

Rhabdoid Tumor of Kidney

This highly malignant renal tumor is unrelated to Wilms' tumor and usually presents in the first few months of life (Weeks et al. 1989). The tumor is genetically related to the other rhabdoid tumors of infancy, with loss of function mutations involving the gene variously called *hSNF5/INI1/SMARCB1/BAF47* (Biegel et al. 1999). Immunohistochemistry for detecting the lack of production of this gene in rhabdoid tumors and the fact that the gene is otherwise widely expressed in normal tissues is a useful diagnostic test (Hoot et al. 2004). This gene appears to act as a classical tumor suppressor gene; a minority of the patients have a germline mutation (Packer et al. 2002), and familial cases are recognized (Lee et al. 2002). This also explains the association of two primary tumors, the renal rhabdoid and a brain atypical teratoid/rhabdoid (AT/RT) tumor, which have been shown to be the two separate tumors and not a metastasis (Kusafuka et al. 2004).

The tumor classically has a discohesive, infiltrative pattern of growth and is recognized by the presence of so-called rhabdoid cells. These have large open nuclei containing a single very prominent eosinophilic nucleolus. Classic rhabdoid cells have large cytoplasmic inclusions consisting of aggregates of whorled intermediate filaments. These give positive staining for vimentin and cytokeratin by immunohistochemistry, and other antibodies may also be positive in a nonspecific manner. Rhabdoid cells may be focally repre-

sented only in a tumor, and the tumor may have a variety of growth patterns. Rhabdoid tumors can resemble other tumors, for example, clear cell sarcoma of kidney (Weeks et al. 1989; Weeks et al. 1991), and conversely other tumors, including mesoblastic nephroma, metanephric stromal tumor, and Wilms' tumors, can focally resemble rhabdoid tumors.

Hypercalcemia is seen in some cases (Weeks et al. 1989). The tumor is highly malignant and aggressive and tends to respond incompletely to chemotherapy before relapse, and is the subject of studies with novel chemotherapeutic regimens (Tekautz et al. 2005).

Ossifying Renal Tumor of Infancy

Ossifying renal tumor of infancy is a rare and apparently benign tumor described in neonates (Jerkins and Callihan 1986; Sotelo-Avila et al. 1995). The tumor is usually partly intracalyceal and may resemble a staghorn calculus attached to the renal medulla. Microscopy shows islands of partly mineralized osteoid separated by polygonal cells and often separate areas of spindle cells resembling blastema or nephrogenic rest.

Other Renal Tumors

Other rare tumors of the kidney include the presentation of acute myeloid leukemia (Butani and Paulson 2003). Clear cell sarcoma of kidney is very rare in neonates (Mazzoleni et al. 2003). A congenital sarcoma exactly resembling clear cell sarcoma of kidney has been described in the terminal ileum (Kataoka et al. 1993).

Liver Tumors

The incidence of metastatic neuroblastoma or leukemia involving the liver exceeds that of primary liver tumors in the first month of life (Isaacs 1997). Vascular tumors, mesenchymal hamartoma, and hepatoblastoma are the only primary lesions seen with any frequency (Dehner 1981). Teratoma (Tapper and Lack 1983), yolk sac tumor, and rhabdoid tumor (Scheimberg et al. 1996) are also described. Rhabdoid tumor associated with brain tumor is also recognized (Chang et al. 1989). Some tumors are difficult to classify (Ohyama et al. 2000). A combined yolk sac tumor and hepatoblastoma has been reported in a 6-month-old infant (Cross and Variend 1992). Choriocarcinoma is also described (Kim et al. 1993). Congenital hepatic adenomas are rare (Werb et al. 1992; Resnick et al. 1995). In later childhood they are associated with congenital disorders such as Fanconi's anemia and glycogen storage disorders (Resnick et al. 1995). Undifferentiated sarcomas may be seen in infancy though it appears to not be reported in a neonate (Bisogno et al. 2002).

Infantile Hemangioendothelioma

Infantile hemangioendothelioma is the most common vascular tumor of the liver in infants, most being diagnosed before 6 months of age. Large tumors present with an abdominal mass, high-output heart failure, hepatic failure, or intraabdominal hemorrhage following rupture during delivery. Occasionally, sequestration of platelets causes a bleeding diathesis (Kasabach–Merritt syndrome). Multiple liver tumors are frequent, and 11% to 40% of patients also have cutaneous hemangiomas. Small tumors may be encountered as an incidental necropsy finding.

The surface of the liver over the tumor may be umbilicated, and the cut surface shows red, brown, or tan, soft or firm tissue often with focal fibrosis or flecks of calcification. Microscopy shows small vascular channels, included bile ducts, extramedullary hemopoiesis, and focal involutionary changes. A study of 91 cases showed that features such as mitotic figures or endothelial budding and tufting (type 2 change) did not predict the outcome (Dehner and Ishak 1971). Poor prognostic features were congestive heart failure, jaundice, multiple tumor nodules, and absent cavernous differentiation (Selby et al. 1994). Most deaths occurred within a month of diagnosis. Spontaneous regression is less common in visceral than in cutaneous hemangiomas but is said to occur in approximately 60% of cases. Steroids and embolization are the treatment modalities (Warmann et al. 2003).

Hepatoblastoma

Hepatoblastoma is the commonest malignant liver tumor of infancy (Weinberg and Finegold 1983) but is rarely found in neonates (Kazzi et al. 1989; Endo et al. 1996), when it has also been described with glomerulocystic disease (Greer et al. 1998). A recent increase in incidence of hepatoblastoma has been attributed to the increased survival of low birth weight babies (Ross and Gurney 1998; Tanimura et al. 1998). There is a definite association with intrauterine growth restriction and maternal preeclampsia (Ansell et al. 2005), and with the familial adenomatous polyposis (Iwama et al. 1994; Hirschman et al. 2005). It occurs in children with hemihypertrophy or BWS, and it has also rarely been reported in siblings, in conjunction with nephroblastoma, in association with maternal use of oral contraceptives, and in fetal alcohol syndrome (Lack et al. 1982). Multiple hepatoblastomas occurred in a patient with trisomy 18 (Teraguchi et al. 1997). Genetic changes in hepatoblastoma include translocations of chromosome 1q12-2 (Tomlinson et al. 2005). Serum α-fetoprotein is usually but not always markedly elevated, and may be differentiated from that produced by yolk sac tumor by subfractionation (Tsuchida et al. 1997).

The histological pattern of hepatoblastoma has prognostic significance. Survival is commoner in children with predominantly fetal tumors (which have a very low mitotic rate) than in those with conspicuous embryonal or mesenchymal components (Haas et al. 1989; Stocker 1994). Variants with an unfavorable prognosis are the anaplastic type, small cell variant, and macrotrabecular pattern (Gonzalez-Crussi et al. 1982; Dehner and Manivel 1988; Haas et al. 2001). The definition for anaplastic cells now uses the same criteria as the Wilms' tumors. Surgical excision is a prerequisite for survival and is possible in approaching 100% of stages I and II patients but only 25% of stage IV patients (Ortega et al. 2000). Pretreatment with chemotherapy may increase the proportion of heterologous elements, particularly osteoid (Saxena et al. 1993), and the response obtained may reflect the prognosis (Heifetz et al. 1997). The cell of origin of hepatoblastoma is unknown, but the hepatic small cell (stem cell) has been suggested (Ruck et al. 1996).

Mesenchymal Hamartoma

Mesenchymal hamartoma is a tumor of infants and young children usually arising in the right lobe of the liver. Of 30 cases reported by Stocker and Ishak (1983), seven patients were less than 3 months old. A few patients had adrenal cytomegaly and pancreatic islet hyperplasia. Mesenchymal hamartoma has been diagnosed antenatally (Tovbin et al. 1997). The microscopic appearance is characterized by progressive expansion of portal tracts by mesenchymal tissue, which becomes confluent, entrapping hepatocytes. Cysts may be lined by biliary epithelium or devoid of an epithelial lining. Enlargement of cystic spaces after birth may lead to rapid growth and suspicion of malignancy. Surgical excision is curative although marsupialization of the cysts is also a treatment option. The pathogenesis is uncertain and a vascular etiology has been suggested (Lennington et al. 1993). A genetic change at 19q13 found in two tumors (Mascarello and Krous 1992; Otal et al. 1994) points to a neoplastic process. Rare reports describe progression to sarcoma, which in one case also showed a 19q13 anomaly) (Lauwers et al. 1997).

Tumors of the Central Nervous System

Congenital tumors of the brain and spinal cord are rare and amount to only 10% of all congenital tumors (Isaacs 2002a,b). Only 1% of childhood brain tumors occur in the neonatal period. Ten percent to 25% of brain tumors diagnosed in the first year of life are evident at birth (Di Rocco et al. 1991, 1993; Isaacs 2002a). The commonest presentation, regardless of tumor type, is enlargement of the head because of hydrocephalus or the mass of the tumor itself. The ability of the neonatal skull to expand enables some of these tumors to grow very large indeed. One in four cases is stillborn, and decompression of the skull may be required to effect vaginal delivery. Choroid plexus tumors may produce hydrocephalus by hypersecretion of cerebrospinal fluid. Occasionally, a hemorrhagic tumor mimics intracerebral

hemorrhage and this possibility should be considered in term infants with unexplained intracerebral bleeding. In children less than 1 year of age the majority of intracerebral tumors are supratentorial, in contrast to the infratentorial position of the majority of tumors in older children (Di Rocco et al. 1991; Bognar 1997).

The commonest congenital brain tumors are teratomas (Buetow et al. 1990; Raisanen and Davis 1993; Isaacs 1997, 2002b). Teratomas usually arise either in the pineal region or in continuity with a pharyngeal teratoma (Pinkerton 1997; Salzman et al. 1997). They are often very large but, with few exceptions, histologically benign. Other tumor types seen in the neonatal period include astrocytoma, medulloblastoma, choroid plexus papilloma and carcinoma (Newbould et al. 1995), atypical teratoid/rhabdoid tumor (AT/RT), medulloepithelioma, ependymoma and ependymoblastoma, craniopharyngioma, suprasellar PNET, and ganglioglioma (Isaacs 2002a).

Medulloblastomas comprises a genetically heterogeneous group of brain tumors composed of small round blue cells showing little differentiation (Ellison 2002). These tumors are highly malignant and may metastasize widely within the CNS. They often show loss of chromosome 22 (Bhattacharjee et al. 1997). Young infants with medulloblastoma have a particularly poor survival (Stiller and Bunch 1992; Katsetos and Burger 1994). Some of these cases have the *INI* mutation and on review are AT/RT, but this does not explain the poor prognosis in this group (Biegel et al. 2000). The desmoplastic variant of medulloblastoma occurs in infants with Gorlin syndrome who carry a mutation of the *PTCH* gene (Amlashi et al. 2003). The large cell/anaplastic variant of medulloblastoma carries a poor prognosis (Giangaspero et al. 1992; Eberhart et al. 2002).

Intracranial rhabdoid tumors—the atypical teratoid/rhabdoid tumors—are described above. There may be only focal rhabdoid change, and the lesions may resemble medulloblastoma. Immunohistochemistry for *INI* is useful. Cytogenetic abnormalities of chromosome 22 may be found in the infant type (Rorke et al. 1995), and the *INI* gene is located on the long arm of chromosome 22 (Biegel et al. 2000). Although generally highly aggressive, some have responded to treatment (Zimmerman et al. 2005). Choroid plexus carcinomas and the AT/RT tumor have overlapping histological features (Judkins et al. 2005; Hasselblatt et al. 2006).

Desmoplastic infantile ganglioglioma is a distinctive supratentorial tumor of infants with a usually favorable outcome. It has a prominent fibrous stroma with neural and astrocytic elements seen with glial fibrillary acidic protein (GFAP) and S100 stains, respectively (Vanden Berg 1993). Although originally thought to be benign, some later cases of a more aggressive type have been described (De Munnynck et al. 2002). Congenital glioblastoma multiforme may have a better survival than in older patients (Winters et al. 2001). The dysembryoplastic neuroepithelial tumor usually presents with epilepsy in infants or older children, and is presumably often present at birth (Daumas-Duport et al. 1988; Prayson et al. 1996). Hypothalamic hamartoblastoma is associated with several syndromes, including the Pallister–Hall syndrome (Squires et al. 1995). Intracranial lipomas are rare and are a maldevelopment usually associated with brain abnormalities, including the absence of the corpus callosum (Bork et al. 1996). They are associated with epilepsy and with Goldenhar syndrome and frontonasal dysplasia.

It is worth noting that the cerebrospinal fluid of infants may be cellular and contain granular cells from the cerebellum that can mimic a small cell tumor (Fischer et al. 1989).

Gonads

Yolk sac tumor is the commonest tumor of the infant testis and is adequately treated by orchidectomy alone if confined to the testis (Ross et al. 2002). Juvenile granulosa cell tumor can have a similar histological appearance, and testis-sparing surgery is to be considered (Shukla et al. 2004). Germ cell tumors of the neonate are biologically and genetically different from those in older patients (Mostert et al. 2000). Occasionally teratoma presents in the infant testis, and is composed of mature elements with or without immature neuroglia. Some benign mucinous cysts in the infant testis probably represent a benign monodermal teratoma. Unlike its counterpart in adolescents and adults, the teratoma behaves in a benign

manner. Sporadic germ cell tumors of the infant testis are not accompanied by intratubular germ cell neoplasia in the absence of gonadal dysgenesis. Gonadoblastoma has been described at birth (Luisiri et al. 1991), and is usually associated with XY gonadal dysgenesis. It has also been described in a phenotypic female with XY karyotype and camptomelic dysplasia (Hong et al. 1995).

Skin Tumors

Skin tumors presenting in the neonate include hemangiomas, neuroblastoma, leukemia, and rhabdoid tumors, as described above. Congenital melanocytic nevii occur in around 1% of newborns, although large congenital nevi (>20 cm in diameter) are very uncommon. The large nevi not infrequently contain nodules, sometimes larger than 1 cm. These may show a variety of appearances with cellular proliferative or hamartomatous appearances, and neural-like patterns that may mimic benign peripheral nerve sheath tumors and other mesenchymal elements like cartilage may be seen. The larger proliferative nodules may mimic melanoma, especially on frozen section (Leech et al. 2004). Some nodules may show genetic changes suggesting malignant progression (Bastian et al. 2002). Congenital melanomas are very rare (Asai et al. 2004) but can be fatal with metastases (Tannous et al. 2005). The risk of malignant melanoma developing in giant nevi is around 2% to 5%, particularly if satellite nodules are present, and most will present by 10 years of age (Swerdlow et al. 1995; Hale et al. 2005; Ka et al. 2005). Giant nevi may be associated with neurocutaneous melanosis and significant morbidity, and also may involve the placenta (Antaya et al. 1995; Hale et al. 2005).

The term *blueberry muffin baby* unfortunately is becoming common for a neonate with multiple blue skin nodules. This can be seen in congenital tumors (e.g., neuroblastoma, leukemia, rhabdomyosarcoma, choriocarcinoma), extramedullary hemopoiesis (due to a cause for fetal anemia such as rhesus disease, infection, or myeloproliferative disorder), histiocytoses (e.g., LCH, Griscelli syndrome), or infection (e.g., cytomegalovirus and rubella), and also is mimicked by vascular tumors (hemangiomas and blue rubber bleb syndrome).

Other Organ-Specific Tumors

The lung is more often the site of metastatic or multifocal disease including hemangiomas and myofibromatosis. Cystic lesions of the lung need careful evaluation. Pleuropulmonary blastoma can be predominantly cystic (type I) through to solid (type III), and the tumors in the older infants and children tend to be more solid and behave in a malignant fashion (Priest et al. 1997; Hill 2005). Useful information is present at the registry Web site (http://www.ppbregistry.org). It has been suggested that all congenital lung cysts should be resected and carefully examined. The tumor is associated with cystic nephroma in the patient or a sibling and a family history of malignancies (Priest et al. 1997). There is an apparent histological overlap with the congenital pulmonary (cystic) adenomatoid malformation (CPAM) especially types 1 and 4 (MacSweeney et al. 2003; Hill 2005). Trisomy 8 is seen in the tumor, but this is not specific (Hill 2005). CPAM has been associated with epithelial malignancies, and atypical goblet cell hyperplasia has been described in infancy (Stacher et al. 2004).

Isaacs (2004) reviewed congenital cardiac tumors. The rhabdomyoma is the most common and is associated with tuberous sclerosis, especially if multiple. Other tumors include the teratoma, fibroma, and oncocytic cardiomyopathy. Myxomas are associated with Carney's triad and Lamb syndrome. Gastrointestinal stromal tumors (GIST) are occasionally reported in neonates (Bates et al. 2000; Yantiss et al. 2000).

Maternal Malignant Disease in Pregnancy

Cancer in pregnant women is commoner than primary malignant tumors in their offspring. Figures for maternal cancer range from 1 per 1000 pregnancies (Potter and Schoeneman 1970; Smith et al. 2003) to 1 per 6000 live births (Haas 1984). The coexistence of pregnancy and maternal malignancy imposes difficult therapeutic dilemmas that are beyond the scope of this chapter (Donegan 1983; Rosenstock 1983). About 60% of the tumors are diagnosed in the 12 months after delivery

(Smith et al. 2003). Fetal and placental metastasis from maternal disease has been reviewed by several authors (Ackerman and Gilbert-Barness 1997; Fox 1997; Benirschke and Kaufmann 2000). True placental involvement with villous invasion has to be distinguished from tumor in the intervillous space, which is more common. Melanoma appears to have a particular propensity to metastasize to the placenta and fetus (Ackerman and Gilbert-Barness 1997; Baergen et al. 1997), although metastatic breast tumor within the fetal blood vessels is well described in several series (Ackerman and Gilbert-Barness 1997). The prognosis for the mother is dismal because placental involvement by cancer indicates disseminated disease, but survival data are historical. Management may be influenced by the supposed risk of metastatic spread of the maternal tumor to the fetus. Available evidence shows that this concern is largely unjustified (Ackerman and Gilbert-Barness 1997). Of the 25 cases of fetal and placental metastasis reported in the 106 years prior to 1973, only 11 involved the fetus (Rothman et al. 1973). Although a wide range of tumor types metastasized to the placenta, melanoma appeared most likely to affect the fetus. Rarely, maternal leukemia, lymphoma, small cell carcinoma of the lung (Teksam et al. 2004), and pulmonary adenocarcinoma (Walker et al. 2002) have been described presenting as a metastasis in the fetus or infant.

The placenta is probably always affected if there are metastases in the fetus. The placenta appears to act as a barrier to maternal tumors, and the fetus may be able to recognize and reject foreign maternal antigens expressed by the tumor. Maternal malignant melanoma metastatic to the fetus has regressed spontaneously after birth, perhaps as a result of a fetal immune response (Rothman et al. 1973). Although fetal tumors such as neuroblastoma may involve the placenta, there is no report of a fetal tumor metastatic to the mother.

Wiztleben and Bruninga (1968) have drawn attention to a rare but characteristic syndrome of infantile choriocarcinoma caused by metastatic placental choriocarcinoma (Witzleben and Bruninga 1968). The young infant develops anemia, hepatomegaly, bleeding, and endocrine abnormalities, such as breast enlargement, with signs of disseminated tumor, especially in the liver and lungs, and sometimes skin nodules (Avril et al. 1986). Evidence of spread of choriocarcinoma to the mother may precede or follow its appearance in the child (Tsukamoto et al. 1986; Picton et al. 1995), or she may be unaffected (Kim et al. 1993). Choriocarcinoma in the placenta may not have been noticed in these cases but may resemble an infarct or cause fetomaternal hemorrhage. Choriocarcinoma of the placenta is occasionally an incidental finding (Fox and Laurini 1988; Sebire et al. 2005). Occasionally, complete hydatidiform mole occurs with a live fetus and is believed to arise from a multiple pregnancy (Matsui et al. 1999). This phenomenon also occurs following assisted conception (Shozu et al. 1998).

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16
The Impact of Infection During Pregnancy on the Mother and Baby

Heather E. Jeffery and Monica M. Lahra

Infection continues to account for a major proportion of maternal, fetal, and neonatal mortality and morbidity worldwide.

In the developing world, maternal systemic infections, such as pneumonia, malaria, tuberculosis, typhoid fever, and pyelonephritis, which are often functions of poverty, crowding, and malnutrition, impose health costs to the mother and risks to the fetus. These risks include spontaneous abortion, stillbirth, preterm labor and preterm birth, low birth weight, intrauterine growth restriction (IUGR), and infection. This is in addition to the rapidly escalating rates of a number of sexually transmitted diseases, in particular, human immunodeficiency virus (HIV) infection with its associated comorbidities.

In the developed world, preterm birth remains a major, unresolved public health issue. Intrauterine infection has been shown to play a major role in induction of preterm birth and neonatal infection (Goldenberg et al. 2000b). More recently the contribution of asymptomatic bacteruria (Rouse et al. 1995; Smaill 2001), periodontal disease (Offenbacher et al. 1996; Newnham et al. 2005), and abnormal vaginal flora (Kiss et al. 2004) to preterm labor and delivery has been recognized.

Congenital Infections

Vertical transmission of infection (from mother to baby) occurs across mammalian species causing injury, malformation, sepsis, and death.

The mode of transmission of infection from mother to fetus includes the hematogenous route, ascending infection from the lower genital tract, and perinatal acquisition, which includes nosocomial infection and transmission of infection via breast milk (maternal or banked milk).

The impact of infection (bacterial, viral, or other) on the mother or the fetus is dependent on maternal and fetal factors in addition to the pathogenic properties of the infecting agent. Maternal factors include immune function and status, anatomical factors, and comorbidity. Infecting agent factors include dose, exposure, and individual virulence factors. Fetal factors include gestational age, developmental stage, and fetal immune function. Table 16.1 summarizes the potential impact on the fetus and neonate with respect to the ante-, peri-, and postnatal periods.

The impact of infection in pregnancy on both mother and baby is discussed in this chapter.

Infection and Preterm Birth

Preterm birth is the leading cause of perinatal death and long-term handicap, accounting for 70% of perinatal deaths in developed countries such as the United States, where approximately 10% of all births are preterm (Goldenberg et al. 2000b).

Preterm births can be categorized into three groups (Arias and Tomich 1982): (1) spontaneous preterm labor and intact membranes; (2) preterm labor with prelabor rupture of membranes; and (3) preterm birth indicated for maternal or fetal reasons. Preterm deliveries that fall into the first

Table 16.1. Impact of congenital infection on pregnancy outcome

Antenatal	Perinatal	Postnatal
Preterm labor	Sepsis	Infection
Fetal injury	Perinatal death	Malformation
Malformation		Developmental abnormalities
Intrauterine growth restriction		Small for gestational age
Intrauterine death		Neonatal death

two categories are most commonly associated with ascending intrauterine infection.

The source of intrauterine infection is most commonly ascending organisms from the lower genital tract and less commonly blood-borne infection (e.g., listeriosis). Uncommonly, retrograde infection via the fallopian tubes or iatrogenic infection introduced from invasive procedures such as amniocentesis occurs (Goldenberg et al. 2000b) (Fig. 16.1).

Ascending infection resulting in chorioamnionitis may account for up to 50% of preterm births at less than 30 weeks' gestation (Lockwood 2002). The organisms implicated are largely anaerobes and genital mycoplasmas, but can include organisms such as group B streptococcus (GBS).

The inflammatory response in chorioamnionitis is predominantly maternal in origin. Chorioamnionitis may be diagnosed by histological or

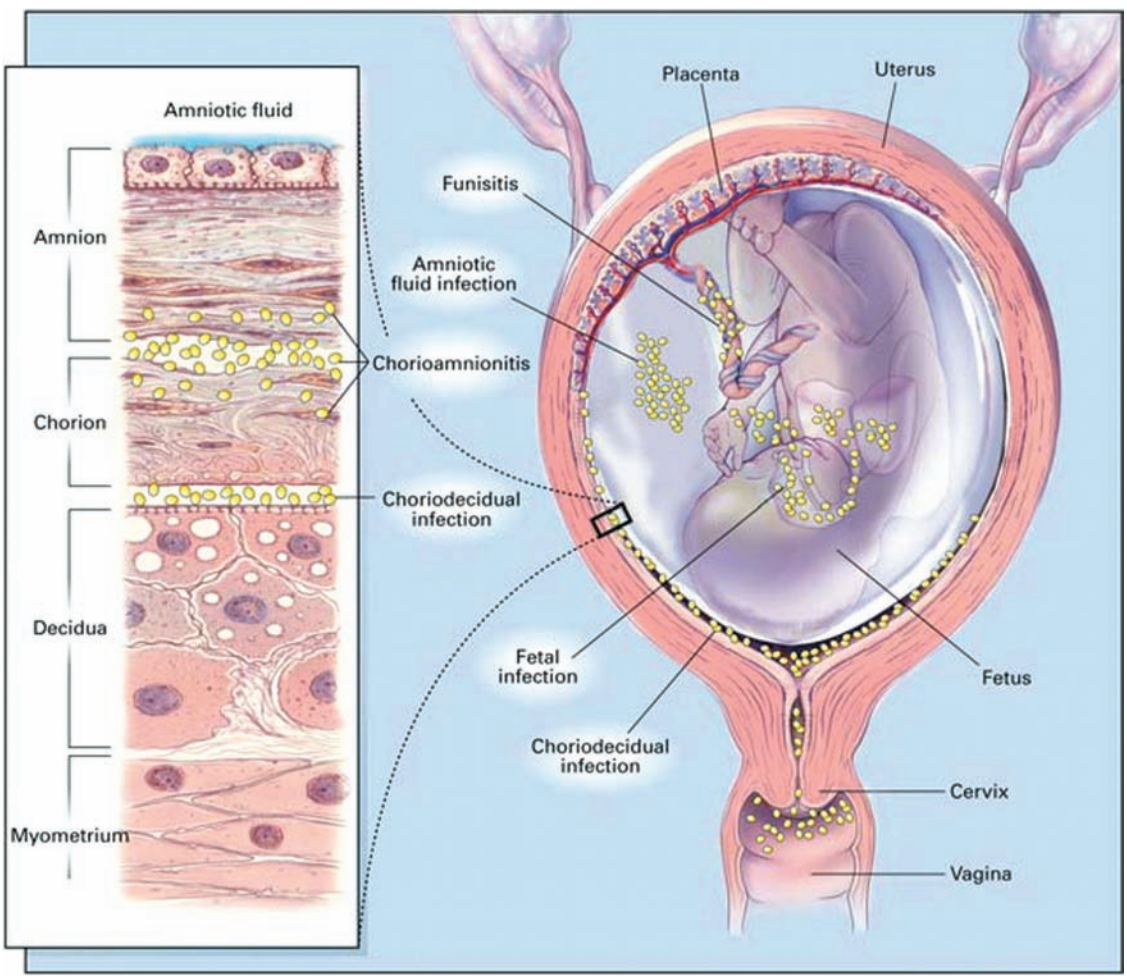

Figure 16.1. Cross section of a gravid uterus illustrating routes of intrauterine infection. (From Goldenberg et al. 2000b, with permission. Copyright © 2000, Massachusetts Medical Society. All rights reserved.)

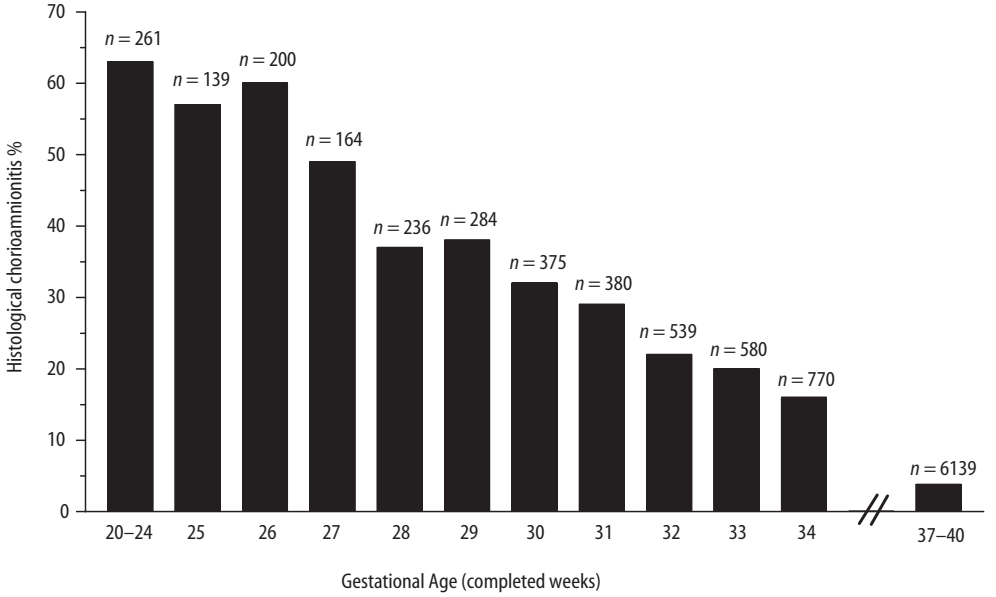

FIGURE 16.2. Incidence of histological chorioamnionitis by gestational age in a large preterm cohort compared with a term cohort. (From Lahra and Jeffery 2004, with permission from Elsevier.)

by clinical findings. The hallmarks of clinical chorioamnionitis are maternal fever, uterine tenderness, and discharge. However, it is important to note that in the vast majority of cases chorioamnionitis is clinically silent and is most often diagnosed histologically. There is a linear and inverse relationship between histological chorioamnionitis and gestational age, and it is most common at gestations less than 30 weeks (Lahra and Jeffery 2004) (Fig. 16.2). The incidence of histological chorioamnionitis at term gestation, using the same methodology and diagnostic criteria, was 3.8% (Russell 1979).

The inflammatory response includes the production of proinflammatory cytokines and inflammatory mediators (prostaglandins, leukotrienes, etc.). The associative evidence suggests that inflammation triggers prostaglandin synthesis in the amnion, chorion, decidua, and myometrium, leading to myometrial contractions and cervical dilatation (preterm labor) with further augmentation of the whole process. Proinflammatory cytokines also stimulate production of matrix metalloproteases by the chorion and amnion, which lead to cervical ripening and membrane rupture (preterm prelabor rupture of membranes) and cervical maturation leading to preterm birth (Romero et al. 2003).

Entry of organisms or bacterial products into the amniotic fluid and subsequently into the fetus similarly provokes elevated fetal proinflammatory cytokine levels in a response known as the fetal inflammatory response syndrome (FIRS). Subclinical fetal infection is underrecognized and is associated with preterm birth, fetal injury, and intrauterine death. It is also associated with infection in the neonatal period. One study found that fetal bacteremia was eight times more likely in women with positive amniotic fluid cultures than those with negative cultures (33% versus 4%) (Carrol et al. 1996).

The fetal inflammatory response is manifested histologically as chorionic vasculitis, umbilical vasculitis, or funisitis (Yoon et al. 2000; Pacora et al. 2002).

The fetal inflammatory response and its importance has been summarized by Leviton et al. (1999):

- The fetus contributes to the cellular inflammatory response in amniotic fluid infection.

- The more severe the histological inflammatory response, the higher the level of cytokines in the amniotic fluid.
- Funisitis is better than membrane inflammation in predicting preterm labor and perinatal death.
- Elevated proinflammatory cytokine levels in the amniotic fluid, umbilical cord blood, and early neonatal blood are some of the best predictors of preterm parturition, cerebral white matter damage, and cerebral palsy.
- Proinflammatory cytokines are elevated in the brains of infants who die with histological evidence of white matter damage.

Chronic Uterine Infection

While intrauterine infection has been considered to be acute, there is evidence suggesting that it may be chronic. Genetic amniocentesis at 16 to 21 weeks identified *Mycoplasma hominis* and *Ureaplasma urealyticum* on culture in a small number of women (Cassell et al. 1983). This clinically silent infection was associated with adverse pregnancy outcome (pregnancy loss and preterm birth) and histological chorioamnionitis. Similarly, in summarizing cytokine findings in midtrimester amniotic fluid, Romero et al. (2003) reported higher median interleukin-6 (IL-6) levels in amniotic fluid of those who delivered preterm compared to those who delivered at term. Maternal IL-6 levels were not associated with adverse pregnancy outcome. In contrast, elevated levels of maternal granulocyte colony-stimulating factor at 24 and 28 weeks' gestation was associated with preterm birth at <32 weeks' gestation but not with late spontaneous preterm birth, in a case-control study of asymptomatic women (Goldenberg et al. 2000a).

Animal models support the human evidence that bacteria or bacterial products (lipopolysaccharides) can precipitate preterm birth. In rabbits and monkeys, intraamniotic injection of a range of bacteria including GBS, fusobacterium species, gram-negative anaerobes, *Prevotella bivia* and *Escherichia coli* lead to rapid onset of labor accompanied by increased cytokine levels and histological chorioamnionitis (Romero et al. 2001; Newnham et al. 2005).

These findings highlight the importance of histological and microbiological examination of the placenta, extraplacental membranes, and umbilical cord in preterm birth, stillbirth, and term babies requiring admission to an intensive care facility. The role of cytokine measurement in clinical practice and prediction of preterm labor is in evolution.

Specific Maternal Infections

Urinary Tract Infection

Background

Urinary tract infections (UTIs) are the most common bacterial infection in pregnancy and occur when bacteria enter and infect the normally sterile urinary tract. Urinary tract infection may be classified as symptomatic bacteruria and asymptomatic bacteruria.

Symptomatic infections may be localized to the bladder (cystitis) or involve kidney infection (pyelonephritis) with systemic symptoms and signs. Urinary tract anomalies, HIV, and diabetes increase the risk of pyelonephritis as does pregnancy (Foxman 2003).

By contrast, asymptomatic bacteruria (ASB) is more common and requires screening midstream urine at the first antenatal visit for detection, preferably at 12 to 16 weeks' gestation (Nicolle et al. 2005). Identification is important as UTI in pregnancy can lead to maternal and perinatal complications (Pastore et al. 1999).

Microbiology

Causative organisms are predominantly gram negative (>90%), most commonly *E. coli* and less often *Enterobacter* species, *Proteus mirabilis, Klebsiella* species, *Citrobacter* species, *and Pseudomonas* species. Gram-positive organisms such as GBS account for about 5% of UTIs in pregnant women (Le et al. 2004).

Epidemiology

Acute pyelonephritis occurs in 1% to 2% of pregnant women, predominantly at around 28 to 30 weeks' gestation, acute cystitis in 1% to 4%,

and asymptomatic bacteruria in 2% to 10% (Le et al. 2004).

Pathogenesis

Factors increasing the risk of UTI include (1) the normally short female urethra, which is exposed to enteric bacteria from the nearby vagina and rectum; (2) ureteral dilatation occurring throughout pregnancy beginning from the sixth week and decreased ureteral and bladder tone, contributing to urinary stasis and ureterovesical reflux; and (3) mechanical compression of the ureters and bladder from the enlarging uterus, leading to urinary stasis (Le et al. 2004). Untreated asymptomatic bacteruria will lead to pyelonephritis in up to 35% of women (Nicolle et al. 2005).

Clinical Features

Typically, cystitis is associated with burning on urination, urgency, frequency, and suprapubic discomfort, although the last two symptoms may also occur as part of advancing pregnancy. Pyelonephritis is often characterized by high temperature and costovertebral tenderness; other symptoms may include chills, nausea, and vomiting.

Diagnosis

On culture of a midstream clean-catch specimen of urine, ASB is defined as $\geqslant 10^5$ colony-forming units per milliliter (CFU/mL), cystitis $\geqslant 10^3$ CFU/mL, and pyelonephritis $\geqslant 10^4$ CFU/mL (Le et al. 2004). Rapid diagnostic tests such as nitrites, urine dipstick, or Gram staining of urine have poor sensitivity and positive predictive values (Bachman et al. 1993).

Treatment

A systematic review has confirmed that treatment of asymptomatic bacteruria significantly improves outcomes for the mother and baby and is cost-effective (Rouse et al. 1995). The odds ratio (OR) and confidence interval (CI) indicate a significant reduction in subsequent pyelonephritis (OR 0.24, 95% CI 0.19–0.32) and preterm or low birth weight delivery (OR 0.60, 95% CI 0.45–0.80) (Smaill 2001).

Initial antibiotic therapy for asymptomatic bacteruria and for cystitis, while awaiting results of culture and sensitivity, should cover *E. coli* and depends on local resistance patterns. β-lactams including cephalosporins and other derivatives are well tolerated, safe, and have minimal maternal side effects. Intravenous penicillin G or ampicillin is recommended for GBS infections (Le et al. 2004). Currently as it is unknown if a 1-day course is as effective as a 4- to 7-day course for asymptomatic bacteruria (Villar et al. 2000), 3 to 7 days of treatment is recommended (Nicolle et al. 2005). A 10-day follow-up culture after completion of the course is recommended.

Both symptomatic and asymptomatic GBS bacteruria require immediate treatment as well as intrapartum antibiotic prophylaxis (Centers for Disease Control and Prevention 2002a). Treatment of UTIs due to GBS significantly reduced preterm rupture of membranes and preterm delivery in the only reported randomized control trial (Thomsen et al. 1987).

Acute pyelonephritis requires hospitalization and urgent treatment to reduce perinatal and maternal complications. Injectable cephalosporins and the combination of ampicillin and gentamycin are usually effective. Other antimicrobial regimens are available (Le et al. 2004). Insufficient data limit recommendation of any specific regimen for symptomatic UTIs in pregnancy (Vazquez and Villar 2003). Close follow-up of UTIs in pregnancy is necessary to prevent complications and to evaluate the need for suppression therapy to prevent recurrences.

Public Health Issues

1. The cost-effectiveness of screening and treatment for asymptomatic bacteruria in pregnancy is justified (Rouse et al. 1995).
2. Group B streptococcus bacteruria in pregnancy, whether symptomatic or asymptomatic, requires intrapartum prophylaxis to prevent early-onset GBS neonatal disease (Centers for Disease Control and Prevention 2002a).
3. A reported possible association between UTI in pregnancy and cognitive delay of the infant emphasizes the importance of health education, screening for bacteruria in early pregnancy, rapid

diagnosis of UTIs, and immediate treatment (Mittal and Wing 2005).

Pneumonia

Background

In Western countries, pneumonia in pregnancy is uncommon despite a potential maternal vulnerability to respiratory infection from the reported decrease in cell-mediated immunity, changes in respiratory physiology, and anesthetic interventions (Lim et al. 2001).

Microbiology

More often than not, the causative organism is unknown, which is in part due to underinvestigation. *Streptococcus pneumoniae* is the most common pathogen isolated followed by *Haemophilus influenzae*. Very occasionally *Mycoplasma pneumoniae, Legionella* species, *Staphylococcus aureus*, influenza virus, and varicella-zoster virus are causative (Lim et al. 2001). Influenza A (types A, B, and C) is usually associated with epidemics and can be particularly severe in pregnancy (Lim et al. 2001).

Epidemiology and Transmission: Incidence, Carriage, and Seroprevalence

The incidence of hospitalization for pneumonia in pregnancy does not seem to differ from that in young, nonpregnant adults (Lim et al. 2001) and has been reported as 1.5 per 1000 deliveries (Laibl and Sheffield 2005b). However, the incidence is much higher in patients with HIV, especially if the $CD4^+$ lymphocyte count is <500/mm, when *Pneumocystis jiroveci* (previously *Pneumocystis carinii*) pneumonia and bacterial pneumonia are more likely (Laibl and Sheffield 2005b).

Risk factors include anemia, smoking, and immunosuppression, whether by prescribed drugs, HIV, illicit drug use, asthma, or chronic respiratory diseases (Berkowitz and LaSala 1990; Lim et al. 2001).

Pathogenesis

The normal host defenses (anatomical barriers, ciliary function, immune function, phagocytic activity) ensure sterility of the lower airways. However, microorganisms can enter the lower respiratory tract by aspiration, inhalation, or occasionally spread from the blood to lung. Pneumonia then occurs if there is a defect in host defenses (e.g., HIV) or the microorganisms are especially pathogenic or profuse.

Clinical Features

Typically the features include dyspnea at rest, cough, and fever, and may include pleuritic chest pain and fine rales on auscultation, but none are definitive. Misdiagnosis (pyelonephritis, appendicitis, preterm labor) or delay in diagnosis in pregnancy has been documented (Yost et al. 2000; Lim et al. 2001).

Diagnosis

Chest x-ray (CXR) is important for diagnosis, although the presence of an infiltrate is not diagnostic of the causative agent. Gram stain of sputum, cultures of sputum and blood, serology, and nucleic acid amplification testing (NAAT) improve the likelihood of identifying the causative pathogen.

Treatment

Antibiotics with a clear safety record in pregnancy for treatment of bacterial pneumonia include penicillins, cephalosporins, and macrolides. The safety in pregnancy of other antimicrobials are listed elsewhere (Lim et al. 2001).

Prevention

Primary prevention of the most common bacterial and viral pneumonias is by vaccination. Prepregnancy counseling should include disease or vaccination history of *S. pneumoniae, H. influenzae* type b, influenza (inactivated vaccine), and varicella. In adults, while systematic review indicates that the pneumococcal vaccine does not appear to reduce the incidence of pneumonia or death, the reports of case-control studies indicate a significant 53% reduction in invasive pneumococcal disease (Dear et al. 2003).

Secondary prevention methods include hand washing, respiratory and contact isolation, and contact prophylaxis.

Public Health Issues

Viral pneumonias, especially influenza and varicella, are readily spread by aerosol in both the community and hospital settings. Identification of these agents as being causative requires special infectious disease precautions, particularly in hospitals. Varicella-zoster immunoglobulin, given within 96 hours of exposure to varicella, can prevent or attenuate the disease and is safe in pregnancy, as is the inactivated vaccine, but the live attenuated vaccine is not safe in pregnancy (Laibl and Sheffield 2005b).

Abnormal Vaginal Tract Flora

Bacterial Vaginosis

Background

Bacterial vaginosis (BV) is an overgrowth of vaginal anaerobes with depletion of the normal lactobacillus leading to an increase in vaginal pH from normal (<4.5) up to 7. The etiology is unclear and BV may be symptomatic or asymptomatic.

Bacterial vaginosis is the commonest cause of abnormal vaginal discharge in women of reproductive age. During pregnancy it is important because of the associated adverse perinatal outcomes including chorioamnionitis, intraamniotic infection, late miscarriage, premature rupture of membranes, preterm birth, and postpartum endometritis. In addition, there is an increased susceptibility to HIV infection if exposure to the virus occurs (Yudin 2005).

Microbiology

The most common organisms implicated in BV are *Gardnerella vaginalis, Mobiluncus* spp., *Bacteroides* spp. and *Mycoplasma hominis* in conjunction with a decrease in the normal population of lactobacilli. The spectrum of organisms is dynamic over the course of BV. Studies using polymerase chain reaction (PCR) methodology suggest a greater complexity than is currently recognized and is one reason for treatment failure (DeVillard et al. 2005).

Epidemiology

The reported carriage rate in women varies widely from 5% to 51%. In the pregnant population, prevalence ranges from 6% to 32%. Risk factors include smoking, sexual activity, douching, and black race (Yudin 2005). The etiology is unknown; however, the epidemiology of BV does have some features of a sexually transmitted infection (STI). Furthermore, findings from a recent cohort study suggest BV is sexually transmitted (Bradshaw et al. 2005).

Pathogenesis

The replacement of the predominant commensal vaginal lactobacilli (normally >95%) with a high concentration of polymicrobes may be due to transmissible lactobacillus phages (Blackwell 1999), which destroy the normal flora with secondary overgrowth of anaerobes.

Clinical Features

The clinical feature is vaginal discharge with a distinctive fishy odor, which is due to polyamines and trimethylamine. Approximately 50% of women are asymptomatic.

Diagnosis

Gram-stained, vaginal smears are examined using either Nugent's criteria or Spiegel's criteria, and estimating relative proportions of bacterial types is most commonly used and is reliable (Yudin 2005). Alternatively, a clinical but more problematic method is used (Amsel's criteria), which requires the presence of at least three of the following: thin, white-gray, homogeneous discharge; pH of vaginal fluid >4.5; fishy odor released on adding alkali (1% or 10% potassium hydroxide), and examination for clue cells on direct microscopy (Yudin 2005).

Treatment

Oral or topical antimicrobials include metronidazole and clindamycin. Screening of partners does not affect the recurrence rate, which can be as high as two thirds of treated women, due to relapse or reinfection (Yudin 2005).

Prevention

Primary prevention by vaccination is not available, but risk can be reduced by limiting factors such as smoking, douching, and number of sex partners.

Screening for BV in high-risk pregnant women (previous preterm birth) and treatment may prevent preterm rupture of membranes and low birth weight. No evidence is provided from the same systematic review that screening and treating low risk women prevents preterm birth (McDonald et al. 2005). This advice is challenged by Lamont et al. (2003) and Lamont (2005), who conclude that, if screening and treatment is performed early in the second trimester in low risk pregnant women, intervention is protective.

Public Health Issues

Further controlled trials are needed to evaluate the role of early treatment of BV in the first or early second trimester in the prevention of preterm birth.

Candidiasis

Background

Candidal vulvovaginitis is a common clinical problem, affecting most adult women at least once during their lifetime. An estimated 75% of women will have at least one episode and 40% will have two or more episodes. Vulvovaginal candidiasis can be classified as uncomplicated or complicated, based on clinical presentation, microbiology, host factors, and response to therapy (Centers for Disease Control and Prevention 2002b) (Table 16.2). *Candida* is a normal commensal flora in humans and is commonly found on the skin and in the gastrointestinal and genital tracts.

Microbiology

Yeasts are unicellular organisms that form part of the commensal flora in humans and are recognized as opportunistic pathogens. *Candida albicans* causes approximately 80% of yeast infections and *Candida glabrata* and *Candida tropicalis* most of the remainder. Laboratory identification to species level is based on growth and morphological and biochemical characteristics.

Table 16.2. Classification of vulvovaginal candidiasis (VVC)

Uncomplicated VVC	Complicated VVC
Sporadic or infrequent	Recurrent
OR	OR
Mild to moderate vulvovaginal candidiasis	Severe vulvovaginal candidiasis
OR	OR
Likely to be *C. albicans*	Nonalbicans candidiasis
OR	OR
Nonimmunocompromised host	Women with uncontrolled diabetes, debilitation or those who are pregnant

Source: Centers for Disease Control and Prevention (2002b).

Epidemiology

Most candidal infections are endogenous, but human-to-human transmission exists (vertical from mother to baby) (Fig. 16.3). Sexual transmission, however, is not a significant factor (Carr et al. 1998). Recurrent vulvovaginal candidiasis, defined as four or more attacks of symptomatic candidal vaginitis in a 12-month period, is estimated at 5% of women during reproductive years (Centers for Disease Control and Prevention 2002b).

Pathogenesis

Pregnancy is one factor that increases susceptibility to vaginal yeast infection, by enhancing the adherence of *Candida* to vaginal epithelial cells, through the effect of estrogen. Other factors include oral contraceptives, broad-spectrum anti-

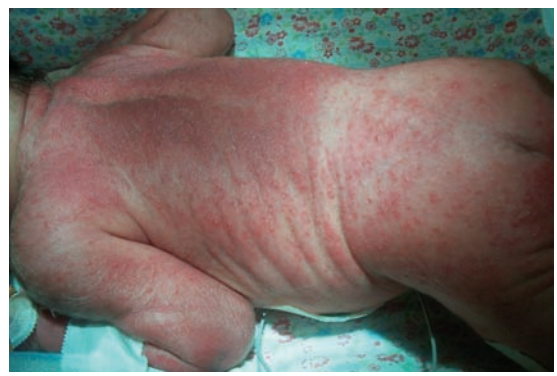

Figure 16.3. *Candida albicans* congenital infection showing pustules, vesicles, and diffuse erythema on day 3. Monochorionic twin died day 1 from septicemia with *Candida albicans.*

biotics, diabetes, systemic steroids, obesity, and immunocompromised states including HIV infection. Stress-induced and premenstrual yeast vaginitis is frequently described by women, but the cause is unknown (Carr et al. 1998).

Recurrent candidal vaginitis can be partly attributed to reduced T-lymphocyte reactivity to *Candida* antigen, thus permitting proliferation and germination that is limited to the genital tract. Relapse rather than reinfection is suggested by negative cultures after treatment and by same-strain Candida-positive cultures at 30 days in 25% to 30% of women (Carr et al. 1998).

Clinical Features

Most women with candidal vulvovaginitis have symptoms. Prominent but nonspecific symptoms are vaginal discharge, pruritus, vaginal soreness, external dysuria, and dyspareunia. The vulva is red, often with scaling and fissures, and the vaginal rugations are red and inflamed with adherent vaginal discharge that is thick, white to yellow in color, and odorless (Carr et al. 1998; Centers for Disease Control and Prevention 2002b).

Candida, although common in the vagina, is a rare cause of amnionitis (Chaim et al. 1992) and an even rarer cause of neonatal skin infection at birth (pustules, vesicles, or diffuse erythema), which is responsive to topical therapy (Fig. 16.3).

Disseminated candidiasis in the neonate is usually limited to the extremely low birth weight/early gestational age infant, typically as a nosocomial, catheter-acquired complication. The meninges, kidneys, and eyes may be involved. There is insufficient evidence from systematic review to recommend prophylactic oral antifungal agents to prevent systemic *Candida* infection in preterm infants (Austin and Darlow 2003).

Diagnosis

Vaginal swab is used for microscopy, culture, and sensitivity. In determining the cause of vaginitis (Table 16.3), the following points are noteworthy:

- Failure to identify the cause of vaginitis by laboratory testing occurs in only a minority of women.
- Culture for *Trichomonas vaginalis* is more sensitive than microscopic examination.
- *Chlamydia trachomatis* and *Neisseria gonorrhoeae* can sometimes cause vaginal discharge in addition to candidal, trichomonal, and chlamydial infections.

Treatment

Topical azole therapy, applied for 7 days, is recommended for candidiasis in pregnancy (Young and Jewell 2001). Treatment of sex partners is not recommended, other than for recurrent infection (Centers for Disease Control and Prevention 2002b).

Prevention

Prevention entails avoidance of factors that increase susceptibility, such as broad-spectrum antibiotics. In the 1980s a trial of *C. albicans* oral vaccine was discontinued, although phase I efforts continue (Fletcher 2001).

Public Health Issues

A large randomized trial found a significant reduction in preterm delivery following screening and standardized treatment for vaginal infections at 15 to 19 weeks' gestation. The most prevalent

Table 16.3. Diagnosis of vaginal infection

Infection	Symptoms	Physical examination	Discharge	Odor	pH	Diagnosis
Yeast infection	Pruritus	Fissures, labial rash	Thick, white to yellow "cottage cheese"	Absent	<4.5	KOH wet prep + hyphae C&S
Bacterial vaginosis	Variable, 50% asymptomatic		Thin, white to gray, homogeneous	Present (fishy)	>4.5	Clinical criteria, 3 of 4 Gram stain
Trichomonas infection	Pruritus, often asymptomatic	Strawbery cervix	Profuse, green watery	Present or absent	>4.5	NS wet prep + trichomonads C&S

C&S, culture and sensitivity.
Source: Modified from Carr et al. 1998, with permission from Blackwell Publishing.

infection was candidiasis (Kiss et al. 2004). This needs confirmation from further trials as the potential impact in the prevention of preterm birth is great.

Sexually Transmitted Infections in Women and Adverse Pregnancy and Neonatal Outcomes

Sexually transmitted infections (STIs), a leading public health problem worldwide, produce a large burden of disease in women of reproductive age. Vertical transmission to the fetus occurs either by ascending infection from the vagina or cervix or by transplacental transmission from infected maternal blood. The estimated number of pregnant women in the U.S. with STIs each year is listed in Table 16.4. Early detection and treatment are important because of the often severe consequences to mother, fetus, or neonate. The bacterial infections are discussed first and viral infections in a later section.

Public health control measures that are common to all STIs with their potentially adverse reproductive health consequences include primary prevention by community education concerning safe sexual practices and high-risk behavior; screening of at-risk women at STI clinics and elsewhere, and routine screening and treatment of patients' sex partners; testing for other common STIs; reporting of cases by health providers to determine epidemiological trends and facilitate targeted control; personal counseling to induce safe sexual behavior including the use of barrier methods, for example, condoms (Stevens-Simon and Sheeder 2005). Current vaccine development is discussed by Fletcher (2001).

Polizzotto (2005), in critically reviewing the literature, concluded that successful behavioral interventions for STI prevention necessitate tailoring it to culture, ethnicity, and gender, employing interpersonal skills training and delivering the intervention to small groups.

TABLE 16.4. Sexually transmitted infections in pregnant women in the United States

Infection	Estimated number of pregnant women infected per annum
Bacterial vaginosis	800,000 (some evidence of STI)
Herpes simplex	800,000
Chlamydia	200,000
Trichomoniasis	80,000
Gonorrhea	40,000
Hepatitis B	40,000
HIV	8,000
Syphilis	8,000

Source: Centers for Disease Control and Prevention (2005i).

Trichomonas vaginalis

Background

Trichomonal infection is one of the most common STIs and has significant health consequences for men and women.

Microbiology

Trichomonas vaginalis is a flagellated protozoan. The parasite is not normally present in the vagina.

Epidemiology

Compared with other STIs, rates are not higher in the young but are evenly distributed across sexually active women of all age groups. Transmission is almost always sexual, and prevalence is highest among women with multiple sex partners and a history of gonorrhoea (Cotch et al. 1991). Transmission rate from infected women to men and vice versa is high, 70% and 80% to 100%, respectively (Soper 2004). The World Health Organization (WHO) estimates *T. vaginalis* infection accounts for almost half of all curable STIs. Prevalence rates are approximately 10%, depending on the population sampled. *T. vaginalis* infection is commonly associated with other STIs especially gonorrhea and may be a marker of high-risk behaviour, and most infected women also have bacterial vaginosis (Risser et al. 2005).

In pregnant women, there is an association with preterm birth, an increased risk of postcesarean endometritis, and an increased risk of HIV transmission and infectivity (Heine and McGregor 1993; Cotch et al. 1997; Soper 2004). Generally, there is a greater risk of tubal infertility and pelvic inflammatory disease (PID), ectopic pregnancy, and increased risk of cervical cancer (Soper 2004).

Pathogenesis

Symptoms develop after an incubation period of up to 28 days following sexual contact. *T. vaginalis* produces epithelial damage and microulcerations.

Clinical Features

Only about half of all women with infection are symptomatic. The most frequent symptoms and signs include excessive yellow/green vaginal discharge, vulvar itching, vaginal/vulvar erythema, vaginal odor, and occasionally a red, erythematous cervix—"strawberry cervix" (Soper 2004). Symptomatic neonatal infection is unusual. Vaginal discharge in neonates is described, and transmission is probably due to exposure to maternal trichomoniasis (Danesh et al. 1995).

Diagnosis

Several diagnostic methods are available. Those with the highest sensitivity (Sn) and specificity (Sp) include culture in Diamond's media (Sn 85–95%; Sp >95%), antigen based point of care testing (Sn 95%; Sp >95%), and NAATs (Sn >90%; Sp >95%) (Soper 2004).

The most commonly used test is a wet mount preparation and detection of motile trichomonads. This method is highly specific (>90%), but sensitivity is only 50% to 60%. Thus a negative result does not rule out trichomoniasis (Soper 2004). Screening for other STIs is indicated.

Treatment

Treatment of symptomatic pregnant women is indicated, but it remains unknown whether this will have any effect on pregnancy outcomes (Gülmezoglu 2002). In asymptomatic pregnant women, uncertainty remains as to whether to treat. One trial reported a higher rate of preterm delivery in the treated group (Klebanoff et al. 2001).

Sex partners require concomitant treatment. Current therapy uses nitroimidazoles as either a stat dose or longer course. It is advisable not to use metronidazole during the first trimester in pregnancy. Resistance is an emerging problem, estimated at 2.5% to 5% in the U.S. (Brocklehurst 1999).

Prevention

Prevention is dependent on reduction in exposure by abstinence or by having single rather than multiple partners, and consistent and correct use of condoms, which possibly reduces acquisition of *T. vaginalis* infection by women (Holmes et al. 2004). There is no vaccine.

Public Health Issues

Trichomoniasis is underdiagnosed and underreported, despite high prevalence rates and the serious consequences of infection for both men and women. In general, problems relate to routine screening that is limited to STI clinics, vaginal wet mount preparations that are used as diagnostic standards despite limited sensitivity, and nonmandatory public health reporting. Public health control measures similar to those for chlamydia and gonorrhea are needed (see below).

Chlamydia trachomatis

Background

Chlamydia is an STI caused by *C. trachomatis* and is one of the most common infections occurring worldwide. It is the most common STI in the U.S., with an estimated 3 million new cases annually (Workowski et al. 2002). *C. trachomatis* causes ocular trachoma, the commonest cause of preventable blindness, and genital tract infection (cervicitis), which can have both maternal and neonatal complications. Adverse outcomes for women include PID, ectopic pregnancy, and infertility, and for neonates, conjunctivitis and pneumonia (Centers for Disease Control and Prevention 2004). The natural history is unknown, but data suggest chronic asymptomatic or persistent infection is frequent, as is reinfection.

Microbiology

Chlamydia trachomatis is an obligate intracellular bacterium with a biphasic life cycle. It belongs to the family Chlamydiaceae consisting of one genus, *Chlamydia*, with three species, *C. trachomatis, C. pneumoniae*, and *C. psittaci* that cause human disease. *C. trachomatis* is the causative bacterium of trachoma, oculogenital disease, infant pneumonia, and lymphogranuloma venereum (LGV).

Epidemiology and Transmission

The disease is most prevalent in adolescents, with a median prevalence of 15% in young, sexually active women. In particular, young age, female sex, and minority race/ethnicity are the most reliable predictors of infection (Risser et al. 2005). The reported rate in women in the U.S. in 2004 was 485 cases per 100,000. Between 4% and 10% of all pregnant women in the U.S. are diagnosed with chlamydia (Centers for Disease Control and Prevention 2004). Transmission is by vaginal, anal, or oral sex with an infected partner, and vertical transmission can occur from mother to baby during vaginal delivery.

Pathogenesis

Organisms initially infect the cervix and urethra and can then spread to fallopian tubes. Primary infection can also occur in the rectum after anal intercourse. The target cells are the squamous/columnar epithelial cells of the endocervix and upper genital tract. The naturally alkaline pH and exposed cervical columnar cells in the adolescent woman increase vulnerability to infection. The histopathology is that of granulomas and microabscess formation. Natural infection confers limited protection (Risser et al. 2005; Stevens-Simon and Sheeder 2005).

Clinical Features

Chlamydia infection is asymptomatic in about 75% of women. If symptoms occur, 1 to 3 weeks after exposure, they include vaginal discharge and dysuria. Signs most commonly include mucopurulent cervical discharge and hypertrophic cervical ectopy (Peipert 2003). Complications include pelvic (upper genital) inflammation causing PID (in at least 10% to 20% cases), infertility, ectopic pregnancy, and chronic pelvic pain (Risser et al. 2005). Chlamydia and other inflammatory STIs increase the risk of acquiring HIV, if exposed to the virus.

The neonate can become infected during birth from an untreated mother, with resulting risk of conjunctivitis (30–50%), nasopharyngitis (15–20%), and pneumonia (5–10%) (Peipert 2003).

Diagnosis

As Chlamydiae are intracellular parasites, swabs rather than exudate is necessary for analysis. Laboratory diagnosis includes the following (Spigarelli and Biro 2004):

1. Nucleic acid probes: Screening has been significantly improved by use of NAATs on urine and self-collected vaginal swab or on an endocervical swab if a pelvic examination is acceptable. Alternative tests include the following:
2. Cytologic examination for intracytoplasmic inclusions (specific for *C. trachomatis* as iodine stains glycogen in the inclusion bodies).
3. Cell culture (e.g., McCoy or HeLa) with isolation of *C. trachomatis determined* by presence of inclusion bodies.
4. Antigen detection by enzyme-linked immunosorbent assay (ELISA) kits or direct immunofluorescence but not as sensitive as culture, especially if few organisms present.
5. Serology is of limited value, as no distinction is shown between current and past infection. Immunoglobulin M (IgM) antibody detection is useful in neonatal infection.

Co-infection with gonorrhea should be considered.

Treatment

Antibiotic treatment is recommended in pregnancy for the infected mother and partner(s) (Workowski et al. 2002; Holmes et al. 2004). Currently azithromycin or doxycycline is recommended for nonpregnant women and oral erythromycin or amoxicillin for pregnant women (Brocklehurst 1999; Peipert 2003).

Prevention

Primary prevention includes personal and community sexual health education to promote future sexual risk reduction. This includes education to ensure consistent and correct condom use, which effectively reduces acquisition of chlamydial infection by women and men (Holmes et al. 2004).

Secondary prevention, through screening programs and treatment of those infected and their

partners, has led to reductions in disease and incident PID by around 50%, with demonstrated cost-benefit and cost-effectiveness (Centers for Disease Control and Prevention 2004). Screening is recommended for all sexually active women of age 25 years or younger and other asymptomatic women at increased risk for infection including pregnant women (Peipert 2003; Centers for Disease Control and Prevention 2004).

Chlamydia DNA vaccines are in the early stages of development, with an attenuated live vaccine increasingly possible (Rupp et al. 2004).

Public Health Issues

Chlamydial infection is largely asymptomatic and of epidemic proportions. It is the most common notifiable disease in the U.S. and can cause severe reproductive sequelae and costly complications—infertility, ectopic pregnancy, and neonatal disease. Clinicians play a crucial role in recognizing, screening (and notifying the proper authorities), and treating Chlamydia infections especially in adolescents, young adults age 25 years or younger, and other asymptomatic women at increased risk of infection.

Gonorrhea

Background

Gonorrhea is the second most commonly reported infectious disease in the U.S. (after chlamydia). Among women gonorrhea is a major cause of PID, which can lead to infertility, ectopic pregnancy, and chronic pelvic pain, and increase the risk of acquiring HIV. Infected neonates develop conjunctivitis and occasionally sepsis.

Microbiology

N. gonorrhoeae is a gram-negative diplococcus. It is a fastidious organism and an obligate human pathogen. The surface structure is similar to other gram-negative bacteria containing an outer membrane, a middle peptoglycan cell wall, and an inner cytoplasmic membrane. Numerous pili extend from the cell surface. *N. gonorrhoeae* can be cultured, and typing methods can be used to track the epidemiology.

Epidemiology

The reported rate of gonorrhea in the U.S. in 2004 was 113 cases per 100,000 population (Centers for Disease Control and Prevention 2004). *N. gonorrhoeae* is transmitted by sexual intercourse with an infected partner, with risks of about 20% female to male per unprotected vaginal intercourse and 50% to 70% male to female per contact. The risk of transmission from an infected mother to her neonate is 30% to 47% (Brocklehurst 1999).

Pathogenesis

Primary infection of cuboidal or columnar epithelial cells is mediated by pili attaching to the mucosal epithelium followed by penetration of organisms through and between epithelial cells within 24 to 48 hours. A marked neutrophilic response ensues with sloughing of the epithelium and development of microabscesses and discharge of pus. Thus incubation is brief and symptoms develop rapidly (Edwards and Apicella 2004).

Clinical Features

In women gonorrhea is often asymptomatic or with only minor symptoms, so medical care is not sought. Symptoms and signs usually occur within 10 days and include vaginal discharge, dysuria, intermenstrual bleeding, and abdominal pain if ascending infection occurs with PID, with or without mucopurulent cervicitis (Bignell 2001).

Gonorrhea during pregnancy has been associated with prelabor rupture of membranes and with preterm delivery (Brocklehurst 1999). Clinical symptoms and signs are unchanged in pregnant women. However, PID is uncommon after the first trimester. Postpartum endometritis and pelvic sepsis can occur.

Neonates are infected during delivery and occasionally by ascending infection before birth, after prolonged rupture of membranes. Neonatal infection causes purulent bilateral conjunctivitis (Fig. 16.4). Occasionally, infection disseminates, causing sepsis, arthritis, or meningitis (Brocklehurst 1999).

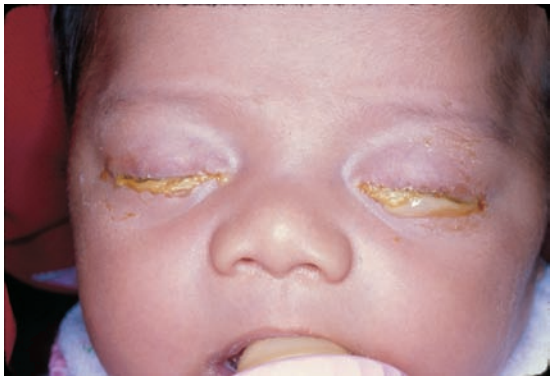

Figure 16.4. Gonococcal conjunctivitis with bilateral, profuse discharge on day 3.

Diagnosis

Endocervical swab and culture on antibiotic-containing selective medium has a sensitivity of 80% to 90%. A NAAT test on the swab is an alternative if viability is in doubt due to delay or alternatively a NAAT test on urine. There is no clinically useful serological test. Co-infection with *Chlamydia* should be considered (Donovan 2004). Eye swabs should be collected in neonates with purulent conjunctivitis for light microscopy, culture, and sensitivity testing. In gonococcal conjunctivitis, after Gram staining, intracellular, gram-negative diplococci can be seen on light microscopy.

Treatment

All tested antibiotic regimens are highly effective in producing microbiological cure with eradication rates of 89% to 97% (Brocklehurst 2002). Penicillin is used for uncomplicated gonorrhea, but alternative antibiotics are recommended for penicillinase-producing *N. gonorrhoeae* (PPNG). Partners should be assessed for treatment.

Prevention

There seems to be little if any natural immunity to gonococcal infection (Hedges et al. 1999). Vaccine development is at an early stage (Fletcher 2001).

Topical or vaginal microbiocides offer intravaginal chemoprophylaxis, before or after intercourse to prevent infection, have a broad spectrum of action, and offer female control. These products are at various stages of development (Rupp et al. 2004).

Health promotion and prevention includes personal and community sexual health education to promote sexual risk reduction, including consistent and correct condom use, which effectively reduces acquisition of gonorrhea by women (Holmes et al. 2004).

In the U.S., eye antibiotics soon after delivery or silver nitrate (1%) aqueous eye drops are recommended for all newborns and are highly effective in preventing gonococcal conjunctivitis in neonates (Centers for Disease Control and Prevention 2002b).

Public Health Issues

Public health control measures include screening of at risk women at STI clinics and elsewhere, and routine screening and treatment of patients' sex partners; testing for other common STIs; and reporting of cases by health providers to facilitate epidemiological trends and targeted control.

Syphilis

Background

Syphilis remains a major cause of adverse pregnancy outcomes in developing countries. Congenital syphilis continues to be a global public health problem, with more than 1 million infected infants born each year, exceeding other neonatal infections such as HIV and tetanus (Saloojee et al. 2004).

Untreated syphilis can lead to serious long-term complications and death. Common to certain other STIs, syphilis facilitates transmission of HIV. Congenital syphilis can cause stillbirth, preterm birth, neonatal death, and neurodisability in survivors.

In the U.S. the rate of primary and secondary syphilis in 2004 was 2.7 cases per 100,000 population. Congenital syphilis declined to 8.8 cases per 100,000 live births, reflecting the reduction in syphilis in women (Centers for Disease Control and Prevention 2004). In contrast, the incidence of congenital syphilis has increased in rural areas of Eastern Europe and central Asia, and high rates of seropositivity are reported in sub-Saharan

Africa at antenatal clinics (3–18%) (Saloojee et al. 2004).

Microbiology

Treponema pallidum are tightly coiled, unicellular, helical organisms. The spirochete cannot be grown on artificial medium and historically has been distinguished under dark field microscopy from nonpathogenic treponemas by the undulating movement about its center. The complete sequencing of the genome of *T. pallidum* is recent, and many of the gene functions in the sequence are as yet unknown (Fraser et al. 1998).

Epidemiology

Transmission is largely due to sexual intercourse. Transmission of infection to the fetus is vertical from an infected mother by either hematogenous spread or direct contact with infectious genital lesions. The likelihood of fetal infection is nearly 100% if the mother has early syphilis characterized by spirochetemia [identified by rapid plasma reagent (RPR) $\geqslant$1:8] and up to 70% four years after the acquisition of maternal disease (Berman 2004; Zeltser and Kurban 2004). Seroprevalence is generally low in high-income countries.

Pathogenesis

T. pallidum either penetrates intact mucosa or enters via abraded skin with rapid spirochetemia and invasion of organs, especially the central nervous system (CNS). The incubation period is dependent on the size of the inoculum, with a median period of 21 days and a range of 3 to 90 days. The host immune response is intense, and the resulting inflammation is responsible for clinical expression. The underlying histological response is an obliterative endarteritis (Zeltser and Kurban 2004).

Untreated syphilis passes through three disease stages: primary, secondary, and tertiary. The primary lesion, the chancre, is a painless ulcer at the site of inoculation, healing after 2 to 8 weeks. The secondary stage occurs at a mean of 6 weeks after contact and consists of parenchymal involvement, constitutional symptoms, and mucocutaneous manifestations with a characteristic maculopapular rash. Primary and secondary stages are contagious. Tertiary disease is symptomatic or unapparent and involves small blood vessels of the aorta, CNS, or both in an obliterative endarteritis as well as a widespread granulomatous lesion called gummas (Zeltser and Kurban 2004).

Clinical Features

The clinical manifestations of syphilis, particularly secondary and tertiary syphilis, are protean (Zeltser and Kurban 2004). Transplacental infection can occur at any stage of pregnancy. More than half of all infected infants are either asymptomatic at birth or have nonspecific signs. Thus suspicion is necessary when positive maternal serology occurs, especially with inadequate or no treatment in pregnancy. Infants of such mothers require treatment.

Early congenital syphilis is characterized by prematurity and low birth weight (10–40%), hepatomegaly with or without splenomegaly (33–100%), generalized vesicular, bullous skin rash initially with mucus patches that then slough (40%), and bone changes of osteochondritis on x-ray (75–100%). None of these are pathognomonic (Saloojee et al. 2004) (Fig. 16.5).

Diagnosis

Key points in testing are summarized below from the more detailed overview by Peeling and Ye (2004).

Serological testing is the mainstay of laboratory diagnosis for primary, secondary, and tertiary

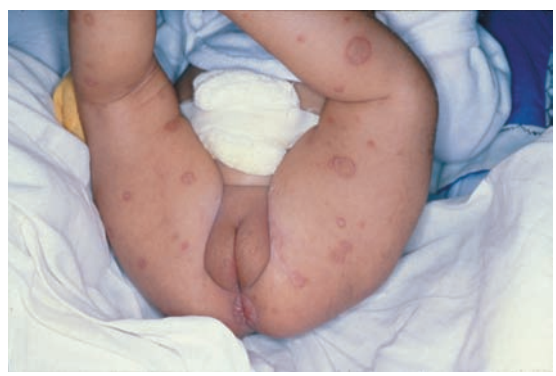

Figure 16.5. Congenital syphilis infection illustrating mucocutaneous rash. Death occurred on day 1 despite treatment.

syphilis. Serological tests are divided into treponemal and nontreponemal tests and neither alone is sufficient for diagnosis.

Nontreponemal tests are used for screening, and they detect antibodies to phospholipid antigens on the treponemal surface. These tests are nonspecific and include the RPR test and the Venereal Diseases Research Laboratory (VDRL) test. They are useful in identifying active infection and monitoring efficacy of treatment. As the nontreponemal tests give false-positive results, confirmation with a treponemal test is needed, such as enzyme immunoassay (EIA), *T. pallidum* hemagglutination assay (TPHA), or fluorescent treponemal antibody absorption test (FTA-ABS). All pregnant women should be screened serologically at least once in pregnancy and at the first antenatal visit (Centers for Disease Control and Prevention 2002b).

Rapid, simple, immunochromatographic treponemal tests that use strips coated with *T. pallidum* antigens on whole blood, serum, or plasma can be used in primary care settings, are simple to use, require no equipment and minimal training, and give a color readout in 10 to 20 minutes.

In the neonate, diagnostic confirmation of congenital syphilis is complicated first by passive transfer of maternal immunoglobulin (IgG), and second by the time taken for detection of IgM antibodies (which do not cross the placenta). Congenital syphilis cannot be excluded by negative tests. Any increased RPR titer compared to the mother is suspicious. Lumbar puncture, cerebrospinal fluid (CSF), and radiography form part of the clinical assessment. However, such investigations in asymptomatic infants in resource poor settings are problematic.

Identification of *T. pallidum* is only possible when patients present with an ulcer, lesion, or rash, and a specimen is collected for dark field microscopy or antigen detection by direct fluorescent antibody to *T. pallidum* (DFA-TP), or *T. pallidum* DNA may be detected by NAATs.

Treatment

T. pallidum has remained sensitive to penicillin for more than half a century and is effective for maternal infection, preventing maternal transmission to the fetus, and treating fetal infection (Centers for Disease Control and Prevention 2002b). Treatment decisions are based on the following steps:

- Identifying syphilis in the mother
- Confirming the adequacy of maternal treatment
- Identifying clinical, laboratory, or radiographic evidence of syphilis in the infant
- Comparing maternal RPR or VDRL titers at delivery with those of the infant

The WHO recommendations are as follows:

1. Asymptomatic infants born to RPR-positive mothers should receive a single intramuscular injection (IMI) dose of 50,000 units/kg benzathine penicillin G.
2. Symptomatic infants receive IMI or intravenous injection (IVI) aqueous crystalline penicillin G, 50,000 units/kg every 12 hours for the first 7 days, then every 8 hours for 3 days (Saloojee et al. 2004).

Prevention

Primary prevention includes programs promoting safe sex including consistent, correct condom use, which will reduce acquisition of syphilis by men and women (Holmes et al. 2004). No vaccine is available.

Secondary prevention depends on screening programs for syphilis in pregnancy with emphasis on providing access to antenatal care early in pregnancy, decentralized services that provide rapid simple test results with appropriate treatment, partner notification and repeat testing in pregnancy, opportunistic testing at sites other than antenatal clinics, and combining programs for prevention of vertical transmission of HIV with that of syphilis (Schmid 2004).

Congenital syphilis can be prevented by detection of maternal infection by the second trimester and appropriate maternal treatment with penicillin.

Public Health Issues

Syphilis, both maternal and congenital, is still a major public health problem worldwide. Screening programs in pregnancy are justified and cost-effective even in low-prevalence areas. The

barriers to achieving this, especially in low-income countries, have been outlined by Schmid (2004) and include recommended interventions at the international, national, and local levels.

Specific Bacterial Infections

Tuberculosis

Background

Tuberculosis (TB) has been documented since antiquity but became epidemic with high mortality in the industrialized world of the 17th and 18th centuries. Tuberculosis became treatable and curable post–World War II with the discovery of streptomycin and then isoniazid. The decline in TB was halted by the HIV epidemic, when coinfection occurred and transmission to others led to a rise in the incidence in the mid-1980s. While pregnancy is not considered to change the course of TB, it does present a risk to the pregnant woman and her fetus (Laibl and Sheffield 2005a).

Microbiology

Tuberculosis is most commonly caused by infection with *Mycobacterium tuberculosis* and rarely to *M. bovis, M. microti, M. africanum*, and *M. canetti*. *Mycobacteria* are aerobic, nonmotile, non–spore-forming bacillus with slow growth in solid media (3 to 8 weeks) compared with liquid broth media (1 to 3 weeks). Typically mycobacteria are acid-fast bacilli (Laibl and Sheffield 2005a).

Epidemiology and Transmission

Humans are the only reservoir for *M. tuberculosis*. In 2000, there were an estimated 8 to 9 million new cases worldwide, most in developing countries with approximately 2 million deaths. The Centers for Disease Control and Prevention (CDC) reported an incidence of TB of 4.9 per 100,000 in the U.S. in 2004 (Centers for Disease Control and Prevention 2005h).

Transmission risk is not altered by pregnancy. Almost all infection is spread by respiratory droplets from coughing, sneezing, talking, and singing. Droplets can remain airborne for hours and, because of the small particle size, can penetrate as far as the alveoli (Frieden et al. 2003). Patients who have smear-positive disease are most infective, those with smear-negative/culture-positive pulmonary disease are less infectious, and culture-negative/pulmonary-disease and extra-pulmonary TB are noninfectious (Laibl and Sheffield 2005a).

Pathogenesis

If droplets containing *M. tuberculosis* access the terminal airspaces, activated macrophages ingest the bacilli, which multiply and lyse the cell. The infection is then either contained at the site of the primary lesion (the Ghon focus) and draining regional lymph nodes, or active disease occurs in the lung or by hematogenous spread to the CNS (meningitis with or without tuberculomas), bones or joints (most commonly the spine, referred to as Pott's disease), genitourinary system (an important cause of infertility in high TB-incidence areas), lymph nodes, pleura, and peritoneum. Cell-mediated immunity appears 3 to 8 weeks after infection. Active disease, uncontained by cell-mediated immunity, is most common in children younger than 5 years of age and in immunosuppressed HIV patients (Frieden et al. 2003). In infected women the incidence of TB peaks at 25 to 34 years (Laibl and Sheffield 2005a).

Clinical Features

In healthy adults infected with TB, 90% to 95% have persistent, asymptomatic, latent TB, and only 5% to 10% develop active TB (Smith 2002). The most common type of TB is pulmonary disease, although 5% to 10% of pregnant women have extrapulmonary TB (Laibl and Sheffield 2005a). Risk factors include HIV, malnutrition, poorly controlled diabetes, and malignant disease. Common symptoms and signs include persistent cough of longer than 2 weeks, fever, night sweats, weight loss, dyspnea, hemoptysis, chest pain, and malaise (Ormerod 2001; Frieden et al. 2003).

Congenital infection is rare (hematogenous or ingested from infected amniotic fluid), and infection is more common after birth from family members with open TB. Neonatal manifestations mimic bacterial or viral infections, unresponsive to usual treatment, in a mother with risk factors for TB. The most common presentation is with

hepatosplenomegaly (76%), respiratory distress (72%), fever (48%), and lymphadenopathy (38%) (Frieden et al. 2003).

Diagnosis

The intradermal administration of tuberculin, standardized, purified protein derivative (PPD-S) assesses cell-mediated immunity to tuberculin, for example, the Mantoux or Heaf test. $CD4^+$ lymphocytes travel to the site, proliferate, and produce cytokines that cause a raised erythematous area, the size of which determines a positive test. This skin test is the only method of reliably detecting *M. tuberculosis* in asymptomatic individuals and is safe in pregnancy (Ormerod 2001). If positive, evidence of disease is assessed by CXR. Tuberculin testing may be negative for months in congenital TB (Laibl and Sheffield 2005a).

Investigations include direct sputum smears, culture, and NAATs. Culture is required for definitive diagnosis and is essential for drug-susceptibility testing (15% to 20% adults with TB are culture-negative) (Frieden et al. 2003). The NAAT testing using PCR is especially valuable and highly predictive for suspicious pulmonary TB that is smear-negative (Frieden et al. 2003). Positive direct sputum smears using the Ziehl-Neelsen method of acid-fast staining are recommended by the WHO, and in high-prevalence, resource-poor areas the smears are considered diagnostic of TB (Schmid 2004).

Treatment

Treatment in pregnancy depends on whether the diagnosis is PPD positive alone, that is, infection but no evidence of active disease (treatment is debatable in pregnancy due to possible isoniazid toxicity), active disease is present (treat), and HIV infected or other risk factors are present (treat). Treatment principles aim to eradicate TB, ensure fewer relapses/failures, achieve higher cure rates, and reduce resistance and include the following (Neralla and Glassroth 2003; Centers for Disease Control and Prevention 2005h):

- Use of multiple drugs to which *M. tuberculosis* is sensitive
- Appropriate drug combinations for a sufficient period of time
- Use of directly observed therapy strategy (DOTS) wherever possible, although a recent systematic review indicates that the effects of direct observation of therapy on treatment completion or cure was similar to self-administered treatment (Volmink and Garner 2003)
- Use of in vitro drug susceptibility and local resistance patterns to guide initial drug choices
- Add multiple not single drugs to a failing regimen
- Emphasize completion of courses

The usual drugs used initially, isoniazid, rifampin, ethambutol, and pyrazinamide, do cross the placenta but are not teratogenic, and women can breast-feed. In addition, pregnant and postpartum women should receive pyridoxine (Laibl and Sheffield 2005a).

Prevention

Prevention and treatment of disease in pregnant women will prevent congenital TB. The Bacille Calmette-Guérin (BCG) vaccine has an overall efficacy of 50% (Colditz et al. 1994) but high protective efficacy against disseminated disease (meningitis and miliary TB) in infants. Advances in vaccine development should lead to more effective vaccines (Haile and Kallenius 2005; Martin 2005).

Public Health Issues

The WHO recommends the five-point DOTS strategy, which achieves average cure rates of 82%, and in HIV areas combine this strategy with effective HIV prevention and cure (Frieden et al. 2003). Effective control is inexpensive and cost-effective. In areas of high incidence and no HIV, case-detection of 70% and cure rates of 85% will lead to a decline in TB of 5% to 10% per year (Frieden et al. 2003).

Group B Streptococcus

Background

Group B Streptococcus (GBS) was recognized as a major cause of bovine mastitis in the 1930s, and emerged as a neonatal human pathogen in the late 1960s. Preventative strategies, based on intra-

partum chemoprophylaxis for maternal carriers (Smaill 1996), in Australia, North America, and some countries in Western Europe have led to up to 80% to 90% reduction in early onset (<48 hours of life) GBS disease (EOGBSD). Most recently, phylogenetic analysis of multilocus sequencing type data has uncovered common ancestry of bovine and human GBS (Bisharat et al. 2004).

Microbiology

Streptococcus agalactiae is a gram-positive coccus that form chains when grown in broth media and exhibit β-hemolysis on blood agar. Defined as Lancefield group B by carbohydrate cell surface antigens, the organism is known as group B β-hemolytic streptococcus.

Epidemiology

The reservoir in humans is the gut. The organism colonizes the lower genital tract of 5% to 40% women and 50% to 75% of their newborns become colonized, but only ~2% acquire EOGBSD. Variance in maternal carriage is due to demographic, endocrine, and behavioral factors, in addition to site swabbed and microbiologic methodology (Jeffery 1996; Bisharat et al. 2004; Gibbs et al. 2004).

Risk factors that have high attack rates (>50/1000 live births) but are relatively uncommon include GBS bacteruria and a sibling with EOGBSD. Risk factors with lower attack rates (>10–25/1000 live births) but more prevalent include heavy vaginal culture at delivery, preterm birth, prolonged rupture of membranes, and intrapartum fever (Benitz et al. 1999).

Neonatal infection can be early onset or late onset (>48 hours and up to 3 months of age); EOGBSD is the commonest cause of early-onset infection in neonates in the developed world. Neonatal infection manifests as pneumonia, bacteremia, and less commonly meningitis (Jeffery 1996).

Pathogenesis

Neonatal GBS infection is most commonly caused by ascending infection via the amniotic fluid. Infected amniotic fluid interfaces with the fetal lungs causing fetal infection and may initiate preterm labor. Less commonly, colonization occurs during delivery, with subsequent infection transmitted via either maternal or nosocomial routes. Neonatal infection is related to absence of maternal type-specific IgG antibodies. The pathogenesis of late-onset GBS disease (LOGBSD) is less well understood and is thought to be due to persistence of oropharyngeal colonization with development of invasive infection or nosocomial transmission of infection. Breast milk transmission of GBS does occur in LOGBSD (Kotiw et al. 2003). Maternal febrile morbidity occurs in 21% of untreated carriers (Boyer and Gotoff 1986).

Clinical Features

Maternal carriers are usually asymptomatic but may develop clinical chorioamnionitis with or without ruptured membranes or a UTI. EOGBSD most commonly presents with respiratory distress or apnea in the term infant, and presentation may be protean in the preterm infant (Jeffery 1996).

Diagnosis

Maternal carriage is diagnosed by culture on selective media of a low vaginal swab. Presumptive identification of GBS is by colony morphology and Gram staining, and definitive identification is by serologic detection. Alternatively, selective media is used for culture of rectovaginal swabs.

The most rapid method of GBS detection is currently by PCR at labor onset; this requires 24-hour laboratory staffing. Initial analysis suggests it would be cost-effective and the most accurate and effective method to determine intrapartum chemoprophylaxis (Haberland et al. 2002).

Neonatal EOGBSD is diagnosed by positive blood culture, CSF, or CXR consistent with infection, supported by a positive urine streptococcal antigen, abnormal white cell count, elevated C reactive protein, or proinflammatory cytokine, for example, IL-6 (Jeffery 1996).

Treatment

Maternal chorioamnionitis and EOGBSD are usually treated with parenteral penicillin and gentamicin (for non–penicillin-allergic mothers) or if allergic an alternative antibiotic (Centers for Disease Control and Prevention 2002b).

Prevention

A screening-based approach to intrapartum chemoprophylaxis is the recommended method of prevention (Centers for Disease Control and Prevention 2002b).

Evaluation of a universal screening approach significantly reduced EOGBSD by >85%, whether performed at 28 weeks' (Jeffery and Lahra 1998) or 35 to 37 weeks' gestation (Puopolo et al. 2005). The decline reported was to 0.22 and 0.37 per 1000 live births, respectively. Recommended prophylactic antibiotic regimens rely on penicillin G or, if not available, ampicillin for nonallergic mothers. For alternative regimens, see Centers for Disease Control and Prevention (2002b) recommendations. Surveillance data for 2004 for the U.S. demonstrates a sustained decline in EOGBSD to 0.34 per 1000 live births (Centers for Disease Control and Prevention 2005d).

Capsular, polysaccharide-protein conjugate, GBS vaccines exist. Significant protective IgG titers in the mother provide passive protection by transplacental transfer. Phase three trials have not yet commenced (Baker and Edwards 2003).

Public Health Issues

Intrapartum chemoprophylaxis for maternal GBS carriers has been very successful in reducing EOGBSD. The most effective prevention is likely to be an adolescent or maternal vaccine (Baker and Edwards 2003; Moore et al. 2003).

Listeria monocytogenes

Background

Listeriosis is the disease caused by *Listeria monocytogenes*. Although an uncommon disease in pregnancy, the incidence of listeriosis is increased in the otherwise healthy, pregnant woman. Most cases in pregnancy are sporadic with occasional outbreaks traced to a common source. Both sporadic and epidemic cases are due usually to contaminated food. Adverse pregnancy outcomes include spontaneous miscarriage, stillbirth, chorioamnionitis, preterm delivery, and neonatal infection. The latter can be early-onset sepsis (vertical ascending or hematogenous transmission) or late-onset meningitis (nosocomial infection) (Silver 1998).

Microbiology

Listeria monocytogenes is a gram-positive motile rod that can be cultivated in the laboratory and has characteristic tumbling motility at room temperature. Specimens from nonsterile sites, such as the vagina, require selective media. Of six species only *L. monocytogenes* is pathogenic for humans.

Epidemiology

The organism is commonly found in soil, dust, processed food, produce, the gut, and feces of domestic and wild animals as well as the human gastrointestinal tract (70% healthy people and up to 44% pregnant women), although positive vaginal swabs are rare, except with perinatal listeriosis. The incidence of listeriosis in pregnant women is 12 per 100,000 compared with 0.7 per 100,000 in the general population (Silver 1998). Transmission is either transplacental or ascending from maternal colonization of the vagina.

Pathogenesis

L. monocytogenes is an unusual bacterium, spreading from cell to cell, avoiding extracellular exposure, and thus bypassing the usual host defenses. Cell-mediated immunity is the primary defense, and as this is depressed in both pregnant women and their neonates, vulnerability to infection is increased. Incubation periods range from 11 to 70 days with a mean of 31 days. Once *L. monocytogenes* crosses the intestinal mucosal barrier, hematogenous spread occurs, especially to the CNS and placenta (Southwick and Purich 1996; Silver 1998).

Clinical Features

Common maternal manifestations, identified in 222 patients with perinatal listeriosis included fever of >38°C (65%), flu-like illness (34%) or asymptomatic (31%), leukocytosis and positive blood (43%) or amniotic fluid culture (8%), and cervical/vaginal culture (34%) or placental culture (11%). Serious maternal illness rarely occurs (Mylonakis et al. 2002). Spontaneous miscarriage or stillbirth occurred in 20%, and 68% of neonates were infected. The most common neonatal manifestations of early listeriosis were respiratory distress (60%), fever ⩾38°C (48%), and neurological

signs with meningitis (25%) (Mylonakis et al. 2002).

Diagnosis

The diagnosis is reliant on suspecting listeriosis in pregnant women with fever or flu-like illness and confirmation of infection with microbiological findings and culture of blood or other sites including placental macroscopic and microscopic findings. In early-onset neonatal infection, *L. monocytogenes* can be isolated from blood, CSF, superficial swabs, placenta, or skin biopsy of a rash.

Treatment

Ampicillin is generally considered the treatment of choice. In those with immune impairment and for all cases of meningitis, dual therapy is recommended. Expert advice should be sought. Successful in utero therapy has been reported.

Prevention

Pregnant women should avoid soft cheeses, unpasteurized milk, and refrigerated ready-to-eat food that is not freshly prepared, for example, delicatessen meats, pates, and salads, and should peel or wash raw fruit and vegetables to remove soil. Hand washing is important. There is no vaccine.

Public Health Issues

U.S. surveys indicate that most pregnant women have limited knowledge of prevention, and hence educational advice both prepregnancy and early in pregnancy is advisable (Ogunmodede et al. 2005).

Surveillance of cases by the CDC in the U.S. and a zero tolerance policy for contaminated foods has seen a 44% decline in perinatal listeriosis (Silver 1998).

Specific Protozoan Infections

Toxoplasmosis

Background

Humans are infected with the protozoan organism *Toxoplasma gondii* by either oral ingestion of the parasite or transplacental transmission. The burden of disease from toxoplasmosis acquired in pregnancy is related not to maternal disease, which is largely asymptomatic, but to vertical transmission to the fetus, with development of congenital toxoplasmosis or sequelae at various times after birth. Prevention is possible but effective treatment of congenital toxoplasmosis is contentious.

Microbiology

T. gondii is an obligate intracellular protozoan with three forms: (1) oocysts (which release sporozoites), formed in the small bowel of cats usually following ingestion of uncooked meat, are excreted in their feces and become infectious in 1 to 5 days; (2) tissue cysts (which contain and may release bradyzoites); (3) tachyzoites, which rapidly divide in macrophages following invasion of the host intestinal wall by either sporozoites from oocysts or bradyzoites from tissue cysts (Kravetz and Federman 2005; Montoya and Rosso 2005).

Epidemiology

Toxoplasmosis is a worldwide zoonosis. Reported rates vary within and between countries. In the U.S. seroprevalence among girls and women who are 15 to 44 years of age is reported to be 15% (Jones et al. 2001). Congenital toxoplasmosis, however, is less common, and the estimated rates from two U.S. surveys varied from 1 to 10 per 10,000 live births, or 400 to 4000 infants per year (Lopez et al. 2000). The incidence depends on primary infection in pregnancy, gestational age at acquisition, and prevention/detection programs. Transmission increases throughout pregnancy, from 6% at 13 weeks' to 40% at 26 weeks' and 72% at 36 weeks' gestation, with less severe consequences to the fetus the later the occurrence of congenital infection in pregnancy. The highest risk of developing early signs such as chorioretinitis and hydrocephaly was approximately 10% when seroconversion occurred at between 24 and 30 weeks' gestation (Dunn et al. 1999).

Risk factors include contact with raw or undercooked meat, and contact with soil as with gardening or eating unwashed vegetables, and contact with infected cat feces.

Pathogenesis

Human and thus maternal infection can occur in three ways: ingestion of tissue cysts in infected undercooked meat; ingestion of infected oocysts through fecal-oral contact; and uncommonly from infected blood to a nonimmune mother. Congenital toxoplasmosis then develops from the transplacental passage of tachyzoites to the fetus (Kravetz and Federman 2005).

The widespread dissemination of *T. gondii* in virtually all cells and tissues is a result of release of tachyzoites from cells with parasitemia via blood and lymphatic routes. Primary maternal infection with parasitemia and before adequate maternal humoral or cellular immunity, combined with high placental blood flow, is conducive to placental transmission being greater with advancing pregnancy (Montoya and Rosso 2005).

Clinical Features

Over 90% of primary infection in pregnancy is asymptomatic if the mother is immunocompetent. Symptomatic infection causes headache, malaise, and cervical lymphadenopathy. Congenital toxoplasmosis is not initially apparent in about 85% of neonates, but chorioretinal and neurological abnormalities develop later. Signs at birth range from mild chorioretinitis to severe early presentation with microcephaly, hydrocephalus, and seizures (Kravetz and Federman 2005).

Diagnosis

As the majority of pregnant women are asymptomatic, diagnosis is difficult and dependent on detecting seroconversion via elevated IgG levels (1 to 2 weeks after infection and elevated indefinitely) or IgM levels (elevated within days, usually for 2 to 3 months, but can remain positive for greater than 2 years) (Kravetz and Federman 2005).

Interpretation of serological tests leads to three possibilities: recently acquired infection with a fetus at risk of congenital disease; infection acquired before pregnancy with an almost zero risk to the fetus of congenital disease unless the mother is immunocompromised; and equivocal results requiring repeat serum samples 3 weeks before or after the initial sample (Montoya 2002; Montoya and Russo 2005).

Prenatal diagnosis is based on PCR testing of amniotic fluid at ≥18 weeks and ultrasound for evidence of calcification or hydrocephalus (Foulon et al. 1999). Neonatal diagnosis is based on serology (IgA and IgM), placental tissue or body fluid culture, PCR of body fluids, and ophthalmological and radiological examination.

Treatment

There are no randomized trials to guide the effectiveness of antenatal treatment with either spiramycin or pyrimethamine-sulfadiazine on risk of congenital infection. Two systematic reviews of observational studies found insufficient evidence on the effects of current antiparasitic treatment compared with no treatment in pregnancy (Peyron and Wallon 2001; Olliaro 2004).

Prevention

Primary prevention by health education at the first antenatal visit for women of child bearing age must include the risks associated with eating undercooked meat, soil-borne transmission, and exposure to cat feces. Hand washing after handling raw meat, cat feces, and soil is important.

Screening for primary *T. gondii* infection in pregnancy is controversial, as the false-positive IgM rate approaches the true positive rate in some countries, and the effectiveness of antenatal treatment for diagnosed maternal infection is uncertain (Peyron et al. 1999; Olliaro 2004). Routine screening is recommended in some countries where prevalence is higher, for example, France and Austria.

Public Health Issues

Health care providers are central to implementing primary prevention. Governments and the meat industry need to continue efforts to reduce *T. gondii* in meat (Lopez et al. 2000).

Malaria

Background

Malaria in pregnancy is a major cause of maternal and neonatal death. An estimated 50 million pregnancies and more than 40% of all births worldwide occur in endemic malarial areas of the tropics

and subtropics (Steketee et al. 2001). Malaria is more common in pregnant than in nonpregnant women, and the risk of adverse maternal and perinatal outcome is greater during first pregnancies and for all gravida women who are HIV positive (ter Kuile et al. 2004). Adverse effects on pregnancy (anemia) and pregnancy outcome [stillbirth, abortion, low birth weight (LBW), prematurity, perinatal mortality] are directly related to the extent of placental malaria and partly to the degree of maternal anemia (Steketee 2003; van Geertruyden et al. 2004). In addition, malaria worldwide is estimated to account for 8% of the 10.6 million deaths in children younger than 5 years of age (Bryce et al. 2005).

Microbiology

Malaria is caused by an intracellular protozoan parasite of the genus *Plasmodium*. Four species infect humans: *P. falciparum, P. vivax, P. ovale*, and *P. malariae*.

Epidemiology and Transmission

Malaria occurs in most tropical regions of sub-Saharan Africa, Southeast Asia, and Latin America. Infected, female, *Anopheles* mosquitoes transmit malaria parasites person to person and are more attracted to pregnant than nonpregnant women (Lindsay et al. 2000). Spread can also occur from transfusion of infected blood or via infected needles.

Pathogenesis

The mosquito releases sporozoite forms of the parasite into the bloodstream, which travel to the liver and invade hepatocytes. Rapid multiplication occurs within the hepatocytes, typically over 1 to 2 weeks, and leads to cell rupture and release of merozoites, which then invade red blood cells. Maturation and division in the erythrocytes occurs over 48 to 72 hours (species dependent), then cell rupture occurs, and new merozoites invade fresh red cells. *P falciparum* is the most lethal malarial parasite, with mortality and morbidity concentrated on pregnant mothers and young children, due to the severity of syndromes such as cerebral malaria, pulmonary edema, and profound anemia (Duffy 2003; Planche and Krishna 2005).

Secondary effects of maternal malaria include suppression of immune responses to vaccination, for example, tetanus toxoid, and reduction in placental transfer of specific antibodies to the fetus, for example, respiratory syncytial virus, measles, and pneumococcus (Duffy 2003).

Placental malaria, characterized by parasitized red cells in placental blood in the intervillous space, is a more common finding than parasites in the peripheral circulation of the mother. Consequences include increased risks of LBW and IUGR, anemia during infancy, and malaria during the early months of life (Fischer 2003).

Clinical Features

Pregnant women in endemic (stable or high transmission) areas are usually asymptomatic but develop anemia, which may be symptomatic depending on the degree. If severe, both maternal morbidity and mortality may be increased. In epidemic (unstable or low transmission) areas, nonimmune pregnant women are at high risk of cerebral malaria, hypoglycemia, pulmonary edema, severe hemolytic anemia, and perinatal death (van Geertruyden et al. 2004). Risk of stillbirth may be increased sevenfold in unstable areas (Newman et al. 2003). Symptoms and signs (fever, chills, headache, sweats, vomiting) are nonspecific. The periodicity of malarial fever is related to the periodic rupture of parasitized red cells. Disease and death occur only during the erythrocytic stages of the parasite and not the liver stage.

Maternal *P. falciparum* infection is one of the most important contributing factors to LBW in first pregnancies and, to a lesser extent, in second pregnancies in Africa, and results in IUGR, prematurity, increased neonatal and perinatal mortality, and infant anemia (Guyatt and Snow 2001, 2004; Fischer 2003; Brabin et al. 2004). Congenital malaria may occur in 7% to 10% of neonates as assessed by malarial parasites in cord blood. Fever, anemia, jaundice, hepatosplenomegaly, and early death may occasionally occur (Fischer 2003).

Diagnosis

Light microscopy of thick and thin Giemsa-stained blood smears is the gold standard for diagnosis.

Rapid diagnostic tests for malaria detect antigens derived from malarial parasites and provide rapid results in 2 to 10 minutes with variable accuracy. Several laboratory tests exist; most accurate and most expensive are tests using PCR to detect parasite nucleic acids. Serology detects antibodies indicating past infection, either by indirect immunofluorescence (IFA) or ELISA.

Treatment

Prompt appropriate treatment of pregnant women with malaria requires early and effective case management in malarial areas together with screening and appropriate treatment of anemia. However, although there is insufficient reliable research on treatment options for malaria in pregnancy (Guerin et al. 2002; Orton and Garner 2005), the WHO (2004b) has outlined recommendations for drug treatment in pregnancy. Morel et al. (2005) concluded that artemisinin-based combination treatment was the most cost-effective strategy for control of malaria in sub-Saharan Africa. The CDC updates treatment options, depending on location, for nonimmune travelers (www.cdc.gov/travel).

Prevention

Nonimmune pregnant women are advised to avoid malaria-endemic areas. In general, chemoprophylaxis is not recommended in areas with fewer than 10 reported cases of *P. falciparum* malaria per 1000 inhabitants per year (Petersen 2004). In endemic areas in Africa, the WHO recommends a triple approach for prevention and control in pregnant women (WHO 2004b):

- Intermittent preventive treatment (IPT) of at least two doses of antimalarial drugs should be given to all pregnant women in areas of stable transmission. The relative risk (RR) of routine chemoprophylaxis (such as sulfadoxine-pyrimethamine) for pregnant women (low parity) in endemic malarial areas indicates significant reduction in severe anemia (RR 0.62, 95% CI 0.50–0.78), LBW (RR 0.55, 95% CI 0.43–0.70), and perinatal death (RR 0.73, 95% CI 0.53–0.99) (Garner and Gülmezoglu 2002).
- Insecticide-treated bed nets (ITNs) are recommended as early in pregnancy as possible and postpartum (WHO 2004b). The ITNs are highly effective in reducing childhood morbidity and mortality from malaria (Lengeler 2004). Their impact is currently being evaluated for pregnant women (Cochrane Infectious Disease group protocol).
- Febrile malaria case management and appropriate treatment.

Public Health Issues

The difficulties associated with mosquito control and drug resistance to the parasite, together with the large burden of disease due to malaria, have provoked intense research for a suitable vaccine. Currently there is no effective, licensed vaccine, although phase III trials are underway (Guerin et al. 2002; Duffy 2003). The global public health need, attributable to the economic and social burden of malaria (Sachs and Malaney 2002), the current situation, and the research and development needs are outlined by Guerin et al. (2002). Research to address the disease burden, in children and pregnant women in particular, and vector control, vaccine development, deployment of rapid tests adapted to field situations, and effective combination drugs are essential priorities to reduce malaria and prevent escalation of the disease (Guerin et al. 2002).

Specific Viral Infections

Parvovirus B19

Background

Discovered by Australian virologist Yvonne Cossart in 1974 (Cossart et al. 1975), parvovirus B19 was not linked with human disease until 1981, when it was associated with acute aplastic crisis in a patient with sickle-cell disease. Parvovirus B19 is now known to cause a range of clinical syndromes in humans dependent on age and immune and hematological status. These range from a very common, mild, or asymptomatic childhood illness to acute aplastic crisis, chronic anemia, hydrops fetalis, and intrauterine death.

Virology

The family is Parvoviride, and the genus is *Erythrovirus*. The genome of parvovirus B19 is very small (*parvum* is a Latin word meaning "small"),

and is composed of single-stranded, linear DNA. Structural features of an icosohedral protein capsid without an envelope make parvovirus B19 resistant to heat and detergent inactivation, and contribute to its transmissibility (Koch 2001).

Epidemiology and Transmission

Parvovirus B19 is ubiquitous and highly contagious (White and Fenner 1994d). Infection with parvovirus B19 is very common in childhood and may be sporadic or epidemic, and occurs most often in late winter and early spring in temperate climates. Children aged 5 to 15 years are most commonly infected, and by adulthood about 60% of people have antibodies. Parvovirus B19 infection is transmitted by respiratory droplets, may also occur via infected blood and blood products, and has been reported in bone marrow transplantation (Heegaard and Laub Peterson 2000). The incubation period is 4 to 21 days.

Pathogenesis

Parvovirus B19 has a limited genome and is therefore only able to replicate in dividing host cells, specifically in human erythroid progenitor cells in the blood, bone marrow, and fetal liver inhibiting erythropoiesis. The cellular receptor for parvovirus B19 is globoside, the P blood group antigen (Brown et al. 1993) in conjunction with a range of β-integrins (Corcoran and Doyle 2004). The P blood group antigen is present on cells of the erythroid lineage. It is also present on cells that are not permissive for replication of parvovirus B19 including endothelial cells, fetal myocardial cells, placenta, and megakaryocytes. The mechanism of damage in these cells is yet to be clarified; however, there is evidence, in myocardial cells at least, that it may be cytokine mediated (Nigro et al. 2004). The tissue distribution of globoside may account for a number of the clinical manifestations of parvovirus B19 infection (Koch 2001). Individuals who lack P antigen are rare and apparently cannot be infected with parvovirus B19 (Corcoran and Doyle 2004).

Clinical Features

The symptomatology of parvovirus B19 infection in the otherwise well host has the following pathophysiological correlates: the initial nonspecific prodromal illness corresponding with viremia (Anderson et al. 1985), which may be absent, very mild, or manifest as fever, malaise, and myalgia. During this time there is destruction of erythroblasts in the bone marrow (White and Fenner 1994d) and a reticulocytopenia without anemia (Anderson et al. 1985). The second stage of rash and arthralgia corresponds with the peak production of specific antibody and are thought to be the result of immune complex deposition.

Importantly, the clinical manifestations of parvovirus B19 vary widely, and infection is frequently subclinical. The commonest clinical presentation, *erythema infectiosum* or fifth disease or "slapped cheek" because of the bright red cheeks the infection causes, occurs in normal, healthy children. Parvovirus B19 can also cause an acute polyarthropathy, usually in adult females. The arthropathy is symmetrical and non-destructive, and may last for weeks. In those with increased erythropoiesis, infection may cause a transient aplastic crisis. In those unable to mount an antibody response, infection may cause chronic anemia (Young and Brown 2004).

Infection in Pregnancy

A seroprevalence of about 60% means that approximately 40% of pregnant women have no immunity. In pregnancy, primary maternal infection can lead to transplacental transmission to the fetus. The transmission rate to the fetus is estimated to be 30% to 50% (Rodis et al. 1990; Koch et al. 1998; Miller et al. 1998). The risk of adverse outcome after parvovirus B19 infection in pregnancy is reported to be up to 9% (Koch 2001). Fetal infection can cause anemia, nonimmune hydrops fetalis, and death.

Hydrops Fetalis

In a study of parvovirus B19 infection in pregnant women, the risk of developing nonimmune hydrops fetalis was found to be about 10% (Yaegashi et al. 1999). Parvovirus B19 infection has been estimated to cause between 10% and 20% of cases of all nonimmune hydrops fetalis (Yaegashi et al. 1994; Jordon 1996). The development of nonimmune hydrops fetalis usually occurs about 2 to 4 weeks after maternal infection as a result of profound fetal anemia and cardiac failure. The pathogenesis of cardiac failure was

once solely attributed to the associated hemodynamic impact of severe anemia; however, infection with parvovirus B19 is now thought also to affect the fetal myocardium (Koch 2001).

Fetal Death

Parvovirus B19 infection is reported to have an estimated risk of fetal death of 5% to 9% (Young and Brown 2004). This has been described in all trimesters, but the period of greatest risk is in the first 20 weeks. Death usually occurs 4 to 6 weeks after infection, not always associated with hydrops (Corcoran and Doyle 2004).

Death in the third trimester has been associated with parvovirus B19. In a prospective, hospital-based, cohort study, 7.5% of all third-trimester intrauterine deaths had detectable parvovirus B19 DNA and were otherwise unexplained (Skjoldebrand-Sparre et al. 2000). Notably, none of these fetuses was hydropic, and the authors suggest investigation for parvovirus B19 infection should be included in the investigation of all intrauterine fetal deaths. Chronic congenital anemia secondary to parvovirus B19 infection has been reported (Brown et al. 1994).

Diagnosis

Parvovirus B19 cannot be grown in standard cell cultures. Diagnosis of infection is made by demonstration of specific anti–parvovirus B19 antibodies. The presence of anti–parvovirus B19 IgM is the best marker of recent/acute infection, and seroconversion from anti–parvovirus IgG negative to IgG positive in paired acute and convalescent sera is also indicative of recent infection (Koch 2001). Nucleic acid amplification tests (NAATs) are available and are useful in tissue. The NAATs are required for diagnosis of persistent infection where antibody production is absent or minimal (Young and Brown 2004).

Treatment

Treatment of parvovirus B19 infection is symptomatic, as there is no specific antiviral therapy. For pregnant women with confirmed infection, ultrasound screening for evidence of fetal edema may be done at 1- to 2-week intervals for 6 to 12 weeks after infection (Schild et al. 1999). Fetal blood sampling under ultrasound guidance may be indicated in the presence of hydrops, and intrauterine transfusion may be offered.

Prevention

Currently there is no vaccine available. Frequent hand washing is recommended to reduce the spread of infection.

Public Health Issues

The CDC does not recommend that pregnant women be excluded from the workplace in a parvovirus B19 outbreak, as this is unlikely to prevent the spread of infection, as those infected are contagious prior to developing the characteristic rash (Centers for Disease Control and Prevention 2005g).

Rubella

Background

Rubella or German measles, also known as third disease, is a benign, mild, self-limiting disease of childhood. In the fetus, in early gestation, infection with rubella can cause profound morbidity and mortality: the congenital rubella syndrome (CRS). In 1941 Australian ophthalmologist Norman Gregg first associated congenital cataract and rubella infection in pregnancy (Gregg 1941).

Virology

The family is Togaviride and the genus is *Rubivirus*. The rubella virus genome is composed of plus sense, single-stranded, linear RNA, with an icosohedral capsid. The virion is spherical and is surrounded by a lipid envelope that is covered with glycoprotein peplomers. Rubella virus is relatively unstable in the environment and is inactivated by disinfectants, solvents, extremes of pH and temperature, and ultraviolet light (Maldonado 2003).

Epidemiology and Transmission

Rubella infection occurs worldwide and is endemic in many areas. In temperate climates infection occurs most commonly in late winter to early spring. During the global rubella epidemic of 1962 to 1965, there were an estimated 12.5 million cases of rubella in the U.S. with 2000 cases complicated

by encephalitis, 11,250 fetal deaths, 2100 neonatal deaths, and 20,000 infants born with CRS. In 1969, live, attenuated rubella vaccines were first licensed in the U.S. and a vaccination program implemented aimed at preventing congenital infection. Since then substantial declines in incidence in the U.S. have been reported and, in 2004, the CDC announced that rubella is no longer endemic in the U.S. (Centers for Disease Control and Prevention 2005a).

A recent survey of WHO member countries found that the number of countries incorporating rubella-containing vaccine into their national immunization schedule increased from 65 (33%) in 1996 to 110 (57%) in 2003 (Centers for Disease Control and Prevention 2005a). Rubella vaccine use worldwide varies by level of economic development: 100% in industrialized countries, 71% in countries with transitional economies, and 48% in developing countries (Robertson et al. 2003). The estimated number of infants worldwide born with CRS each year is more than 100,000 (Robertson et al. 2003).

Infection is spread person to person via respiratory transmission. Humans are the only known reservoir. The virus replicates in the nasopharynx and regional lymph nodes, with viremia occurring 5 to 7 days after infection. Transplacental transmission of infection to the fetus occurs during viremia. The incubation period is 12 to 23 days (average 14 days). Infants infected with rubella in utero can shed the virus for up to 12 months or longer (Cooper and Krugman 1967).

Pathogenesis

Maternal viremia infects the placenta, with subsequent fetal infection. Fetal infection can affect all organs; its impact is most severe in very early pregnancy, coincident with organogenesis. Rubella is teratogenic and causes fetal damage via two mechanisms: a generalized, progressive, necrotizing vasculitis, resulting in parenchymal hypoplasia; and cellular deletion through mitotic arrest and apoptosis (Plotkin 2001; Centers for Disease Control and Prevention 2005a).

Clinical Features

Rubella is asymptomatic in up to 50% cases. Its commonest presentation in children and adults is a rash and lymphadenopathy of nasopharyngeal and upper respiratory tract nodes. Complications of rubella infection include arthropathy (in up to 70% women), and, rarely, thrombocytopenic purpura (more common in children), encephalitis, orchitis, and neuritis.

Infection in Pregnancy

Infection in the first trimester of pregnancy has the most severe consequences for the fetus: malformation, miscarriage, and intrauterine death. Up to 90% of babies infected in the first 11 weeks of gestation develop CRS, the risk declining rapidly after the first trimester, becoming negligible after 16 weeks (Miller et al. 1982). Rubella infection later in gestation does not result in CRS (Centers for Disease Control and Prevention 2001).

Rubella reinfection may occur. It is more common after vaccination than is infection. The risk of CRS after rubella reinfection is extremely small (Banatvala and Brown 2004).

Congenital Rubella Syndrome

Congenital rubella syndrome results from vertical transmission of rubella and typically manifests in infancy. The CDC case definition for CRS is based on clinical and laboratory findings (Centers for Disease Control and Prevention 1997). Infants with CRS usually present with more than one of the following clinical findings; however, they may have only a single defect, most commonly hearing impairment (Centers for Disease Control and Prevention 1997):

a. Cataracts/congenital glaucoma, congenital heart disease (most commonly patent ductus arteriosus or peripheral pulmonary stenosis), hearing impairment, pigmentary retinopathy
b. Purpura, hepatosplenomegaly, jaundice, microcephaly, developmental delay, meningoencephalitis, radiolucent bone disease (Centers for Disease Control and Prevention 1997).

Congenital rubella syndrome is categorized as follows:

1. *Suspected CRS:* not meeting criteria for classification as a probable case but having some consistent clinical findings

2. *Probable CRS:* any two findings from entry "a" (above) or one from "a" and one from category "b" in the absence of any other etiology
3. *Confirmed CRS:* clinically consistent and laboratory confirmed (Centers for Disease Control and Prevention 1997).

Cases are further classified by importation status, and these guidelines should be consulted for further details (Centers for Disease Control and Prevention 1997).

A global review of CRS sequelae from prospective studies with laboratory-confirmed infection found hearing impairment in 60%, congenital heart disease in 45%, microcephaly in 27%, cataracts in 25%, low birth weight (less than 2500 g) in 23%, hepatosplenomegaly in 19%, purpura in 17%, mental retardation in 13%, and meningoencephalitis in 10% of infants with CRS (Reef et al. 2000). The spectrum of clinical findings, including delayed manifestations that may not appear until early adulthood or adulthood, are summarized in Table 16.5. Some developmental defects such as hearing impairment may not become apparent for months or even years (Banatvala and Brown 2004). The most common delayed onset disease in CRS is the development of type 1 diabetes mellitus in adulthood. Follow-up of a cohort of Australians with CRS at age 60 years found a higher prevalence of type 2 diabetes, thyroid disorders, early menopause, and osteoporosis compared with the Australian population (Forrest et al. 2002).

TABLE 16.5. Clinical features of congenital rubella syndrome

Type of defect	Clinical feature
Ocular	Cataracts (uni- or bilateral)**
	Glaucoma**
	Pigmentary retinopathy**
	Microphthalmia
	Iris hypoplasia
	Cloudy cornea
Auditory	Sensorineural deafness (uni- or bilateral)**
Cardiovascular	Persistent ductus arteriosus**
	Pulmonary artery stenosis**
	Ventricular septal defect**
	Myocarditis
Central nervous system	Microcephaly*
	*Meningoencephalitis**
	Psychomotor retardation
	Behavioral disorders
	Speech disorders
	Progressive rubella panencephalitis
Intrauterine growth restriction	
*Thrombocytopenia, with purpura**	
*Hepatitis/hepatosplenomegaly**	
*Bone lesions**	
Pneumonitis	
Lymphadenopathy	
Diabetes mellitus	
Thyroid disorders	

*Commonly recognized in the neonatal period.
**Commonly recognized in early infancy.
Early transient features are *italicized*.
Source: Adapted from Banatvala and Brown (2004).

Diagnosis

Rubella infection is subclinical in up to 50% cases. It is confirmed serologically by detection of rubella-specific IgM or demonstration of a significant rise in rubella-specific IgG in paired acute and convalescent sera by standard serologic assay. Rubella virus may be cultured from a clinical specimen (e.g., blood, nasopharyngeal fluid, urine, or CSF), with the best results from throat swabs. Rubella virus can also be detected by NAATs such as reverse-transcriptase PCR (Centers for Disease Control and Prevention 2001). Molecular typing of rubella virus isolated in culture or NAAT products gives important epidemiological information about the virus. Diagnosis of rubella in pregnancy requires expert consultation given the gravity of the implications. Expert advice should be sought from the nearest rubella reference laboratory regarding specimen collection, transport and handling, and the applications and limitations of the tests available.

Laboratory diagnosis of congenitally acquired rubella and CRS can also be made serologically, by viral culture or by NAATs. Recommendations for testing and interpretation are available (Centers for Disease Control and Prevention 2001).

Treatment

There is no specific antiviral therapy for rubella.

Prevention

Routine determination of immune status to rubella in all women of childbearing age and

vaccination of the nonimmune and nonpregnant is recommended. There is a theoretical risk to the fetus after vaccination in pregnancy; women should be counseled prior to vaccination to avoid pregnancy for 3 months. Early antenatal screening for rubella immune status should be done in all pregnant women. The pregnant nonimmune should be advised to avoid persons with a rash illness and be vaccinated postpartum (Centers for Disease Control and Prevention 2001). Infants with CRS can shed virus for up to 12 months or more and thus pose an infectious risk.

Public Health issues

Rubella and CRS are reportable diseases in the U.S. The CDC recommendations for the elimination of rubella and the prevention of CRS include maintenance of high immunization rates among children and the vaccination of women of childbearing age, in particular those born outside the U.S. Other recommendations are continued surveillance and rapid response to any rubella outbreak (Centers for Disease Control and Prevention 2005a).

Cytomegalovirus

Background

Cytomegalovirus (CMV) infection is of particular significance in the immunocompromised, where it causes severe multisystem disease, and in the fetus, where CMV is now the commonest viral cause of congenital abnormality since the introduction of widespread rubella vaccination.

Virology

The family is Herpesviride, and the subfamily is Betaherpesvirine. Cytomegalovirus is the fifth and the largest of the eight human herpes viruses. It has a double-stranded DNA genome with an icosohedral capsid, surrounded by a lipid envelope that is covered with glycoprotein peplomers.

Epidemiology and Transmission

Cytomegalovirus is ubiquitous and infection is endemic. Seroprevalence varies demographically and geographically, with up to 100% seroprevalence in young adults in developing countries compared with approximately 50% seroprevalence in early adulthood in middle and upper socioeconomic groups in developed countries, with higher rates in certain groups such as those with lower socioeconomic status and those working with young children (Griffiths et al. 1985). Primary CMV infection in pregnancy may result in vertical transmission to the fetus. In the developing world, where seroprevalence of CMV is very high, the impact of congenital CMV is unknown. Thus congenital CMV infection is currently a problem of the developed world.

Congenital CMV infection affects 0.2% to 2% of the 4 million infants born in the U.S. annually, resulting in 10,000 to 80,000 congenitally infected children (Hollier and Grissom 2005).

Transmission of CMV occurs after contact with infected body fluids such as saliva, genital secretions, and urine. Acquisition of infection commonly occurs in infancy especially in a child care setting and, in young adulthood, transmitted by infected saliva and urine during kissing and sexual activity. Cytomegalovirus infection can also be transmitted via infected blood and tissue in transfusion and transplantation.

Vertical transmission of CMV is largely transplacental. Less commonly, perinatal transmission of CMV occurs after contact with infected maternal genital secretions or infected breast milk.

Following resolution of initial infection CMV, like all human herpes viruses, has the capacity to establish latency principally in the salivary glands and renal tubules. Once infected, the host carries the virus for life and may intermittently shed virus in saliva, urine, semen, cervical secretions, or breast milk (White and Fenner 1994a). Reactivation of infection occurs both in healthy and immunosuppressed individuals, and, in pregnancy, may lead to vertical transmission.

Primary CMV infection in pregnancy poses a risk of transmission to the fetus of 30% to 40% (Stagno et al. 1986), whereas the risk of vertical transmission in recurrent CMV infection is between 0.2% and 1.8% (Demmler 1991). Of those infected, 10% to 15% will be symptomatic at birth. The mortality rate of symptomatic babies is 20% to 30%, and 90% of surviving babies will have severe neurological sequelae.[169] More severe sequelae are seen in those children whose mothers

had primary CMV infection (Hollier and Grissom 2005).

Children with congenital CMV who are asymptomatic at birth may have abnormalities evident later in life in 10% to 15% cases, in particular sensorineural hearing loss (Reynolds et al. 1974).

Infection in the first half of pregnancy is more often associated with symptomatic disease in the newborn, whereas infection in the second half of pregnancy is more often initially asymptomatic (Hollier and Grissom 2005).

Pathogenesis

Cytomegalovirus replicates in the nucleus of host cells causing cytomegalic cells, the histological hallmark of CMV infection. Cytomegalic cells are swollen with an enlarged nucleus, which is distended by a huge inclusion separated from the nuclear membrane by a nonstaining halo, giving them the appearance of "owl's eyes." The presence of these cells is indicative of active infection, but they are not always found.

In pregnancy CMV is transmitted in infected leukocytes via the placenta, to the fetus (Trincado and Rawlinson 2001). In the fetus, CMV replicates in the epithelium of the renal tubules and is excreted via urine into the amniotic fluid, where it is reingested by the fetus and the sequence repeated (Hollier and Grissom 2005).

The pathogenesis of fetal cellular injury in congenital CMV is not well understood. There is controversy over whether the virus is directly teratogenic or if its impact is a result of impaired perfusion after vascular injury, as the virus is known to infect endothelium (Schleiss 2005). The CNS is the major target for cellular injury, and this is reflected in extensive neurodevelopmental sequelae.

Clinical Features

Generally a mild, or asymptomatic, nonspecific illness in the otherwise well, CMV can cause a spectrum of clinical illnesses in the both the healthy and immunocompromised. In young adults CMV infection can cause a mononucleosis-like illness that may be difficult to distinguish from Epstein-Barr virus infection. In the immunocompromised, CMV infection causes pneumonitis, gastrointestinal disease, and retinitis, which have significant morbidity and mortality.

Congenital Cytomegalovirus

The early clinical findings in symptomatic congenital CMV may include intrauterine growth restriction and preterm delivery, petechiae, hyperbilirubinemia, hepatosplenomegaly, hepatitis, jaundice, microcephaly, chorioretinitis, ventriculomegaly, periventricular calcifications, and seizures. Late findings include developmental delay, mental retardation, seizures, and sensorineural hearing loss (Hollier and Grissom 2005).

Diagnosis

Congenital CMV is diagnosed by viral detection, for example, viral culture or NAAT of blood, urine, or tissue, in the first 3 weeks of life. In addition, serology for anti-CMV specific IgM in the neonate may be done. Prenatal diagnosis is possible but complex and, when indicated by maternal serology or ultrasound findings, includes amniotic fluid or chorionic villus sampling for CMV detection (culture and NAATs) and quantitation or percutaneous umbilical blood sampling for detection of virus or anti CMV IgM (Enright and Prober 2004; Hollier and Grissom 2005). Increased sensitivity of amniotic fluid testing has been reported when testing is done after 21 weeks' gestation or at least 6 weeks after maternal primary infection (Enders et al. 2001). Collection of fetal blood, amniotic fluid, or chorionic villus sampling is associated with a risk of pregnancy loss, and in active maternal infection, a risk of iatrogenic fetal infection (Hollier and Grissom 2005).

Treatment

Currently, no proven, effective treatment exists for CMV infection in pregnancy. Antiviral drug therapy with ganciclovir in symptomatic neonates with CNS disease has been recently evaluated in a multicenter, randomized, controlled trial, and findings suggest this prevents hearing deterioration, but many subjects were lost to follow-up (Kimberlin et al. 2003). Given the potential toxicity of long-term *ganciclovir* therapy, the Committee on Infectious Diseases of the American

Academy of Pediatrics (CIDAAP) advises additional study before a recommendation be made (American Academy of Pediatrics 2003a).

Prevention

Vaccines are in development though not in clinical use. The CDC recommendations for pregnant women include good personal hygiene, in particular hand washing with soap and water after contact with saliva or diapers. Pregnant women who become ill with a mononucleosis-like illness should be evaluated for CMV infection and counseled regarding risks to the baby. Serology can be performed to determine immune status. Detection or recovery of CMV from urine or vaginal secretions is not an indication for operative delivery. Breast-feeding is not contraindicated in maternal infection, and children should not be screened or excluded from school if they are shedding CMV (Centers for Disease Control and Prevention 2005c).

Public Health Issues

Women of childbearing age who work with infants and children, and who have not previously been infected with CMV, are at potential risk of infection as CMV infection is common in children in day care centers. Since it may be contracted from contact with infected body fluids, particularly saliva and urine, the CDC recommends education regarding CMV and its transmission, and practices such as hand washing, which reduce the risk of infection (Centers for Disease Control and Prevention 2005c).

Varicella Zoster Virus

Background

Varicella zoster virus (VZV) is the third of the human herpes viruses. Infection with VZV causes varicella (chickenpox). Like all human herpes viruses VZV remains latent after infection and reactivation causes herpes zoster (shingles). Primary VZV infection in pregnancy can have severe consequences for both the mother and the baby. Nosocomial transmission of varicella is well recognized (Centers for Disease Control and Prevention 1996).

Virology

The family is Herpesviride, and the subfamily/genus is Alphaherpesvirine/*Varicellovirus*. The Alphaherpesvirine subfamily includes herpes simplex virus 1 (HSV-1), HSV-2, and VZV. The VZV virion is spherical and enveloped with an icosohedral capsid and linear double-stranded DNA genome. The envelope of VZV is studded with glycoproteins. The Alphaherpesvirine all grow rapidly, cause lysis of infected cells, and establish latent infection in the ganglia of sensory nerves. Reactivation triggers replication and shedding of infectious virus (herpes zoster), which is less common than reactivation with HSV (White and Fenner 1994e).

Epidemiology and Transmission

Varicella zoster virus is a common disease of childhood worldwide and 90% of cases occur in children under 13 years of age. By the age of 15 years about 90% of people are immune (Whitely 2000). The implementation of the varicella vaccination program in the U.S. has achieved a reduction in varicella infections in all ages, with the most marked decrease in the 1 to 4 years age group (Jumaan et al. 2005).

Varicella zoster virus is a highly contagious infection that is transmitted person to person by contact, aerosol, or droplet from vesicular fluid of skin lesions, or by infected respiratory tract secretions. Varicella enters the body via the respiratory tract (Centers for Disease Control and Prevention 1996).

A large prospective study of 1373 women with varicella in the first 36 weeks of pregnancy found all cases of congenital varicella occurred at less than 20 weeks' gestation with an overall risk of about 1%. The highest risk (2%) was observed between 13 to 20 weeks (Enders et al. 1994).

Pathogenesis

After infection occurs, VZV replicates initially in the respiratory and oropharyngeal mucosa. The virus then disseminates via the lymphatic system and the bloodstream and replicates in mononuclear leukocytes and capillary endothelial cells. The rash is a result of viral replication in epithelial

cells of the skin (White and Fenner 1994e). The rash is pruritic and characterized by successive crops of macules, papules, and vesicles. Virus is abundant in the characteristic vesicles. The incubation period is typically 14 days.

Clinical Features

Varicella is a rash illness usually acquired in childhood. Adults, adolescents, and the immunocompromised usually have more severe disease and are at increased risk of complications (Centers for Disease Control and Prevention 1996). Complications include secondary bacterial infection of rash lesions, pneumonitis, hepatitis, meningoencephalitis, cerebellar ataxia, and Reye's syndrome.

Varicella Pneumonitis

Infection in pregnancy is associated with an increased risk of pneumonitis. Clinically, respiratory symptoms develop about 4 to 5 days after the onset of the varicella rash and include tachypnea, dyspnea, cough, and fever (Whitely 2000). Radiologically there are bilateral, diffuse, peribronchial nodular infiltrates (Hollier and Grissom 2005). Radiological and clinical findings may be disparate, with abnormal radiology in the absence of abnormal clinical findings (Whitely 2000; Hollier and Grissom 2005). Varicella pneumonitis can be life threatening when it occurs in the second or third trimester of pregnancy (Whitely 2000).

Congenital Varicella

Clinical features of congenital varicella syndrome include cutaneous and skeletal defects with cicatricial skin scarring and limb atrophy. Neurological and ocular defects also occur and include microcephaly and cortical atrophy, seizures, mental retardation, chorioretinitis, microphthalmia, and cataracts. In severely affected infants, neurogenic bladder, hydroureter, hydronephrosis, and severe gastroesophageal reflux are common (Arvin 1996).

Neonatal Varicella

Maternal varicella rash developing up to 5 days before to 2 days after delivery has an attack rate in the newborn of approximately 20% with a mortality rate of about 30% (Preblud et al. 1985). This window of increased vulnerability is due to the time required for the production and transplacental passage of maternal VZV IgG antibodies to the fetus (Miller et al. 1989).

Diagnosis

Confirmation of infection is possible by demonstration of seroconversion or serologic rises using standard assays and acute and convalescent sera. Diagnosis can also be made by isolation of the virus in cell culture, detection of viral nucleic acid in CSF, and detection of viral antigen from scrapings of cutaneous lesions using a direct fluorescent antibody stain (Whitely 2000).

Treatment

The CIDAAP does not routinely recommend acyclovir for pregnant women with uncomplicated varicella because the risks and benefits to the fetus and mother are unknown. For the pregnant patient with serious complications of varicella intravenous acyclovir is recommended (American Academy of Pediatrics 2003d).

Prevention

Live attenuated varicella vaccines were introduced in the U.S. in 1995, and widespread introduction of childhood vaccination has led to a reduction in the incidence of varicella. Varicella vaccine is contraindicated in pregnancy. Postexposure prophylaxis with varicella-zoster immune globulin (VZIG) is recommended for nonimmune pregnant women within 96 hours of exposure (Centers for Disease Control and Prevention 1996) and where VZV immune status is unknown and unable to be determined within this time period (Hollier and Grissom 2005). The administration of VZIG to nonimmune pregnant women has not been shown to prevent viremia or vertical transmission and its consequences, and is given to prevent complications in the mother (Centers for Disease Control and Prevention 1996). Recommendations for administration of VZIG to neonates include those whose mothers have signs and symptoms of VZV infection 5 days before up to 2 days after delivery, neonates who are preterm (less than 28 weeks), and neonates who weigh

1000 g or less at birth regardless of maternal history because these infants may not have maternal antibody (Centers for Disease Control and Prevention 1996). Guidelines should be consulted.

Public Health Issues

Infection control measures recommended for neonates born to mothers with varicella include airborne and contact precautions in addition to standard precautions. These should be maintained until 21 days of age, or 28 days of age if they received VZIG. For exposed nonimmune patients the precautions above are recommended for 8 to 21 days after onset of rash in the index patient and for 28 days in those who received VZIG (American Academy of Pediatrics 2003d).

Herpes Simplex Viruses 1 and 2

Background

Herpes simplex virus 2 and less commonly HSV-1 cause genital herpes. Maternal herpes infection in pregnancy can be transmitted to the neonate to cause neonatal herpes. The estimated annual incidence of neonatal HSV infection in the U.S. is 1 in 1400 to 1 in 30,000 deliveries, resulting in 1500 to 2200 infected babies per year (Enright and Prober 2004).

Virology

The family is Herpesviride, and the subfamily/genus is Alphaherpesvirine/*Simplexvirus*. The Alphaherpesvirine subfamily includes HSV-1, HSV-2, and VZV. The HSV virion is spherical and enveloped with an icosohedral capsid and a linear double-stranded DNA genome. The envelope contains many different glycoproteins. The Alphaherpesvirine all grow rapidly, cause lysis of infected cells, and establish latent infection in the ganglia of sensory nerves. Reactivation triggers replication and shedding of infectious virus (White and Fenner 1994b).

Epidemiology and Transmission

Herpes simplex virus 2 is a sexually transmitted disease and the most common cause of genital ulcer disease worldwide and the most common cause of neonatal herpes. The prevalence of HSV-2 is increasing worldwide but varies widely and is generally higher in developing countries. Prevalence is higher in urban than in rural settings, and varies with age, gender (generally higher in females), ethnicity, sexual practices, and the presence of other sexually transmitted infections.

In the U.S. the overall seroprevalence of HSV-2 is 21% in adults, but is higher among African Americans, who have a seroprevalence of more than 45%, as compared to whites, who have a seroprevalence of approximately 17% (Fleming et al. 1997). Many European countries report a seroprevalence of HSV-2 of less than 15%. In developing countries there is a wide range (2% to 74%) of seroprevalence of HSV-2 reported (Nahmias et al. 1990; Wagner et al. 1994). In sub-Saharan Africa and the Caribbean, many countries have seroprevalence rates of around 50% (Weiss et al. 2001). Data are scarce from Asia and South American countries.

Orolabial HSV-1 infection is a common infection of early childhood. Reactivation of orolabial infection causes cold sores. Virus is shed in these lesions, which are infective. Herpes simplex virus 1 genital infection can occur via autoinfection or by orogenital sex.

Genital Herpes

Genital herpes is caused by HSV-2 and HSV-1. Genital herpes is transmitted sexually when virus-infected secretions come into contact with susceptible mucosal surfaces or breached epithelium. The risk of infection is affected by age, race, number of lifetime sex partners, duration and frequency of sexual intercourse, and income (Mertz et al. 1992).

After infection, HSV may be intermittently shed from the genital tract of both men and women in the absence of prodrome, symptoms, or lesions. In pregnancy, rates of HSV shedding at delivery have been reported at between 0.3% to 0.5% (Brown et al. 1986, 2003) with higher rates of 1.4% reported in women with a history of recurrent HSV infection (Arvin et al. 1986).

Herpes simplex virus infections are defined serologically as primary, nonprimary, and recurrent. Serological primary infection with HSV-1 or HSV-2 occurs in someone who is seronegative to both. Nonprimary infection is defined as

confirmed infection with HSV-1 or HSV-2 that occurs in the presence of heterologous antibody (e.g., infection with HSV-2 in a HSV-2 seronegative person who has antibody to HSV-1). Nonprimary infection is generally clinically milder (Hill and Roberts 2005). These classifications have implications for risk of vertical transmission.

Primary HSV infection in pregnancy confers the highest risk of neonatal infection. The chance of vertical transmission is greatest when maternal infection with HSV-1 or HSV-2 occurs close to the time of labor, with a risk of 50% in primary infection and 33% when infection is a nonprimary first episode (Hill and Roberts 2005). Genital herpes infection acquired in the first half of pregnancy or reactivation of genital HSV acquired prior to pregnancy is associated with a much lower risk (1% to 5%) of vertical transmission (Hill and Roberts 2005).

Neonatal Herpes

Maternal herpes simplex virus infection in pregnancy can be transmitted to the neonate. Maternal HSV infection can be asymptomatic or symptomatic, primary or recurrent disease, and the virus is intermittently shed after infection. More than 85% of neonatal herpes infection occurs after perinatal exposure of the baby to HSV (HSV-2 in about 70%) in infected maternal genital secretions. In about 70% of cases this is a result of asymptomatic HSV shedding near the time of delivery (Whitely et al. 1988).

The rate of recurrence of HSV is higher in pregnant women compared with nonpregnant women and is more common with HSV-2 than HSV-1 (Hill and Roberts 2005).

Approximately 10% of neonatal herpes is acquired postnatally after close contact with family or hospital staff with orolabial or cutaneous HSV infection (most commonly HSV-1) (Enright and Prober 2004). Transplacental infection of herpes virus may rarely occur in women who have primary HSV infection in pregnancy but is extremely rare after recurrent disease (Hill and Roberts 2005).

Pathogenesis

After transmission, HSV replicates locally in epidermal cells of skin or mucous membrane before transit of the virus by retrograde axonal flow up the peripheral sensory nerve fibers to the corresponding sensory ganglia in the brainstem or spinal cord where, thereafter, the viral genome persists indefinitely. Reactivation of infection occurs at any time and can be triggered by stress, ultraviolet light, fever, nerve injury, or immunosuppression. Following reactivation replication occurs in the latently infected neurons, and virus is transported down the axon to the periphery where it multiplies in epithelial cells in the same vicinity as those infected originally (White and Fenner 1994b).

Clinical Features

Neonatal herpes is classified into three subgroups, which have diagnostic, therapeutic, and prognostic implications:

1. Disease localized to the skin, eye, or mouth (SEM)
2. Disease localized to the central nervous system (CNS)
3. Disseminated disease with or without CNS involvement

Neonatal HSV becomes symptomatic within the first month of life, a large proportion having onset in the first week (Hill and Roberts 2005).

The largest study of neonatal HSV found that no single constellation of presenting symptoms and signs identified all cases. Skin vesicles and, in babies with CNS disease, seizures, were among the most suggestive findings of neonatal HSV infection. Other common signs and symptoms were lethargy, fever, conjunctivitis, disseminated intravascular coagulation, and pneumonia. Importantly, skin vesicles were absent in 17% of babies with SEM disease, 32% of babies with CNS disease, and 39% of babies with disseminated HSV disease (Kimberlin et al. 2001).

Ocular involvement can affect the anterior or posterior orbit, can be unilateral or bilateral, and can progress over several weeks and cause cortical blindness (Nahmias 2004). Disseminated disease has the highest mortality and morbidity; CNS disease has a lower mortality but significant morbidity; and SEM has both low morbidity and low mortality when specific antiviral therapy is used (Hill and Roberts 2005).

Diagnosis

Herpes simplex virus infection must be considered in a neonate with mucocutaneous vesicles, or evidence of clinical sepsis with negative cultures at 48 hours with or without associated hepatitis, or disseminated intravascular coagulation, seizures, or pneumonitis (Freedman et al. 2004). Diagnosis is confirmed by isolation of the virus in viral cultures from skin vesicles, nose, throat, or conjunctival swabs or occasionally from CSF; by detection of viral DNA using NAAT of CSF, blood, or skin lesions; or by detection of viral antigen using a direct fluorescent antibody testing of cutaneous lesions. Diagnosis of CNS HSV infection may be difficult, as CSF pleocytosis is not always present and a negative NAAT does not exclude the diagnosis. Neurological imaging studies may be normal early in disease (Freedman et al. 2004).

Treatment

The CIDAAP recommends parenteral acyclovir as the treatment of choice for all neonates with HSV infection, regardless of manifestations and clinical findings. Management of asymptomatic infants exposed to HSV during delivery includes viral cultures of urine and stool; rectal, oral, and nasopharyngeal swabs; and close observation. Treatment should be initiated if cultures are positive or in the presence of clinical signs. Symptomatic, exposed infants should be evaluated and treated immediately. Investigations include swabs, blood, and CSF collected for culture and NAATs (see above). Treatment should be initiated if virus is detected, CSF findings are abnormal, or HSV infection is otherwise strongly suspected (American Academy of Pediatrics 2003b). Ophthalmologic opinion should be sought in any suspected or confirmed case (Nahmias 2004).[0]

Prevention

There is no vaccine in clinical use. The CDC recommendations include abstinence from sexual activity when symptoms or lesions of herpes simplex infection are evident (Centers for Disease Control and Prevention 2005e). Use of condoms can reduce sexual transmission of infection (Centers for Disease Control and Prevention 2005i). In the presence of active genital HSV disease at time of delivery, cesarean section has been shown to prevent neonatal herpes (Brown et al. 2003). Prevention of primary infection in pregnancy is paramount. The CIDAAP recommends that scalp monitoring be avoided if possible in infants of women suspected of having active genital herpes (American Academy of Pediatrics 2003b).

Public Health Issues

From a public health perspective, in developing countries in particular, HSV-2 is implicated in facilitating HIV transmission. In most regions with escalating HIV rates, HSV-2 is highly prevalent (WHO/UNAIDS/LSHTM 2001).

Human Immunodeficiency Viruses

Human immunodeficiency virus 1 (HIV-1) and HIV-2 are the causative agents of acquired immunodeficiency syndrome (AIDS). The viruses are distinct with only 40% homology.

Infection with HIV-2 accounts for a very small proportion of the total HIV infections and is rare in the developed world.

Global data for 2004 show the number of people living with HIV has escalated to an estimated 39.4 million—the highest level ever (WHO 2004a). This report shows the most marked increases in infection in East Asia, Eastern Europe, and Central Asia. In East Asia the incidence increased by 50% over the period 2002 to 2004, a figure that is largely attributed to China's expanding epidemic.

Sub-Saharan Africa, however, remains the worst affected region with an estimated 25.4 million people (including 1.9 million children) living with HIV, and 2.3 million attributable deaths in adults and children in 2004. Women and girls are increasingly affected and now account for just less than half the total estimate and 57% of the proportion infected in sub-Saharan Africa (WHO 2004a). Most HIV-infected children contract HIV from their mothers during pregnancy, delivery, or lactation.

Human Immunodeficiency Virus 1

Virology

The family is Retroviride, and the genus is *Lentivirus.* Retrovirus virions have a diploid, linear,

single-stranded RNA genome with three major structural genes (*gag, env*, and *pol*) as well as several other genes. The genome-nucleoprotein complex is closely associated with molecules of the viral enzymes: reverse transcriptase, integrase, and protease. The virus is enveloped with glycoprotein peplomers (White and Fenner 1994c).

Transmission

Human immunodeficiency virus is transmitted from person to person sexually via infected genital secretions or blood in contact with the rectal, oral, or vaginal mucosa, via infected blood (direct contact with infected blood, transfusion of infected blood or blood products, or contaminated equipment). Transmission occurs vertically from mother to baby and accounts for approximately 90% of HIV infections in children. The estimated rate of vertical transmission of HIV-1 is 30%, with higher rates reported in women who are symptomatic or who have previously delivered an infected baby (Ahmad 2005). The importance of various modes of transmission of HIV-1 differs according to region; in most developing countries the predominant mode of transmission is via heterosexual sex, and vertical transmission is more common than in industrialized countries (Grant and De Cock 2001).

Multiple viral, immune, and clinical factors in both mother and baby influence vertical transmission. The strongest predictors of risk are maternal plasma viral load, obstetric factors, and breastfeeding (Moodley and Moodley 2005). Vertical transmission of HIV can be interrupted and the incidence has been vastly reduced in the developed world using intervention strategies. However, in developing countries vertical transmission is responsible for an estimated 90% of pediatric HIV infections. In addition, the rapidly increasing incidence of HIV in women and girls tragically forebodes that pediatric infections will escalate correspondingly.

Pathogenesis

The specific mechanisms of mother-to-infant transmission of HIV are not understood. However, it is known that transmission may occur transplacentally, during delivery through contact with infected maternal blood and genital secretions, and postnatally after exposure to the virus in breast milk (Ahmad 2005).

Clinical Features

Human immunodeficiency virus disease in infants progresses more rapidly than disease in adults, and most infants develop symptoms within the first few months of life (Ahmad 2005). Clinical manifestations include failure to thrive, generalized lymphadenopathy, hepatomegaly, splenomegaly, parotitis, oral candidiasis, recurrent diarrhea, cardiomyopathy, hepatitis, CNS disease (including developmental delay), recurrent bacterial infections, opportunistic infections, and malignancy (American Academy of Pediatrics 2003c).

Opportunistic infections with *Pneumocystis jiroveci* (previously known as *Pneumocystis carinii*) (Stringer et al. 2002) causing *Pneumocystis* pneumonia or PCP, can occur as early as 4 to 6 weeks of age. Other opportunistic infections include CMV infection, chronic or disseminated HSV or VZV infection, and candidal esophagitis. Less commonly mycobacterium infections and chronic enteritis and rarely disseminated CNS *Toxoplasmosis gondii* or cryptococcal infections occur (American Academy of Pediatrics 2003c).

The development of opportunistic infections, wasting, progressive neurologic disease, high viral load, a low $CD4^+$ T-cell count, and the onset of symptoms in the first year of life are associated with a poor prognosis (American Academy of Pediatrics 2003c).

Diagnosis

Definitive diagnosis of HIV infection in the neonate is made by detection of the virus by nucleic acid testing or viral culture. U.S. guidelines for diagnosis of HIV infection in pregnancy recommend testing within the first 48 hours of life, then at age 1 to 2 months and at 3 to 6 months. Exposed infants testing negative in the first 48 hours of life may be tested at 14 days, which may detect early infection acquired in the peripartum period (National Institutes of Health 2005). A positive test should be confirmed by collecting and testing a second specimen. Human immunodeficiency virus infection is diagnosed by two separate positive HIV virologic tests on two

separate blood samples. Serological testing for diagnosis of HIV infection is not used in the neonate as transplacental passage of maternal antibody occurs. Serology is recommended after 12 months to confirm that maternal antibody has disappeared, and if still seropositive, the infant should be retested at 18 months of age (National Institutes of Health 2005).

Treatment

Current guidelines for the treatment of HIV infection in the U.S. are available online at http://www.aidsinfo.nih.gov.

Prevention

There is no vaccine available. Strategies to prevent vertical transmission are continually evolving. The most recent guidelines for the U.S. are available online (National Institutes of Health 2005). Current recommendations include universal antenatal counseling and screening with consent for HIV-1, the use of antiretroviral therapy for both the infected mother antepartum and intrapartum and her infant postpartum. In addition to antiretroviral therapy for the exposed infant, prophylaxis against *P. jiroveci* commencing at 4 to 6 weeks of age is recommended (Centers for Disease Control and Prevention 1995). Consideration should be given to delivery by elective cesarean section in certain circumstances. The CDC recommends that HIV-positive mothers do not breast-feed to further minimize risk of infection. In the developing world, however, where children are at increased risk of other infectious diseases and nutritional deficit, the benefits of breast-feeding may outweigh the risks (U.S. Department of Health and Human Services 2000).

Public Health Issues

Human immunodeficiency virus infection is a public health crisis in the developing and the developed world. Optimal strategies for the prevention, diagnosis, and therapy of HIV infection are continually under review. Strategies for the prevention of mother-to-child transmission in both the developed and developing world are paramount and are the focus of global public health initiatives.

Human Immunodeficiency Virus 2

Background

Human immunodeficiency virus 2 (HIV-2) was first isolated from a patient with AIDS in West Africa in 1986 two years after the discovery of HIV-1; both belong to the Lentoviride family.

Epidemiology and Transmission

Infection with HIV-2 is concentrated in West Africa, Angola, and Mozambique and accounts for a very small proportion of total HIV infection. Human immunodeficiency virus 2 is rare in the developed world. It is transmitted in the same way as, but less commonly than, HIV-1. Co-infection with HIV-1 and HIV-2 occurs. Human immunodeficiency virus 2 infection in children is rare, and the rate of vertical transmission is reported to be approximately 1% (Grant and De Cock 2001).

Clinical Features

Human immunodeficiency virus 2 infection is associated with opportunistic infection and AIDS, although immunodeficiency seems to develop more slowly and to be less severe (Centers for Disease Control and Prevention 2005f). The typical course of HIV-2 infection from seroconversion is unknown. A recent review of the literature on the natural history of HIV-1 and HIV-2 infections in adults in Africa concluded that, from the limited studies available, those infected with HIV-2 appeared to have a longer and possibly more variable survival than those with HIV-1 (Jaffar et al. 2004).

Diagnosis

Diagnosis of HIV-2 is made by confirmation of a positive HIV-2 specific screening assay by one or two different tests specific for HIV-2. Western blot is commonly used as a confirmatory test, and guidelines for interpretation of Western blot assays vary by organization.

Treatment

The optimal therapeutic strategy for HIV-2 is unknown. Genetic variation in the reverse transcriptase and protease genes of HIV-1 and HIV-2 result in varying susceptibility to the different

classes of antiretroviral agents, in particular the nonnucleoside reverse transcriptase inhibitors. Recommendations from a recent review are, in the absence of large clinical trials of therapy for HIV-2, to combine the experience of management strategies for HIV-1 with the available in vitro susceptibility data for HIV-2 and the evidence from case series to guide management decisions (Hightower and Kallas 2003).

Prevention

Strategies aimed at preventing HIV-1 infection should be effective in the prevention and control of HIV-2 infection (see above). Blood donations are screened for HIV-1 and HIV-2 virus.

Public Health Issues

The CDC recommends continued surveillance for HIV-2 in the U.S. population, as there is the potential for further spread of HIV-2, particularly among intravenous drug users and those with multiple sex partners.

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17
Iatrogenic Disease

Allan G. Howatson

Iatrogenic injury is a feature of all medical practice but it is perhaps nowhere more accepted as an unavoidable consequence of therapy than in obstetric and neonatal medicine. Classical obstetric iatrogenic pathology has been with us since time immemorial, and despite the recognition of causal factors remains a not infrequent occurrence (Ennis and Vincent 1990; CESDI Fifth, Sixth, Seventh and Eighth Reports 1998–2001, www.cemach.org.uk).

The development of invasive antenatal investigation and treatment and the increasingly complex interventions in neonatology have resulted in the appearance of new types and patterns of pathology.

The role of the pathologist in the investigation of perinatal and neonatal death is central to the monitoring of iatrogenic pathology and brings with it considerable responsibilities in the light of potential medicolegal consequences and the need to recognize new problems. It is vital that the pathologist be well versed in the identification of iatrogenic lesions and record with great care unusual findings in cases where novel therapeutic modalities are being employed. Iatrogenic lesions may be of varying degrees of clinical significance. Many, perhaps the majority, are minor and accepted as a consequence of intervention, while others represent serious complications or medical mishaps or reflect poor clinical judgment. Perinatal autopsy examinations provide a vital opportunity to monitor any potential teratogenic effects of drug therapy. In addition, the ability to keep very ill babies alive in neonatal intensive care has resulted in the maturation or evolution of pathological processes in various organs, resulting in the development of new patterns of pathology that need to be recorded and explained.

It is clear that in order for pathologists to contribute effectively to the investigation of perinatal and neonatal deaths and to the understanding of iatrogenic pathology, they require full access to obstetric and neonatal records before they begin the examination. All medical devices, for example, cannulae, should be left in situ prior to the postmortem examination. If these conditions are met, the pathologist can contribute markedly to the improvement in the quality of obstetric and neonatal care.

Maternal Medication During Pregnancy

The recognition that maternal drug therapy poses risks to the fetus at all stages of development has been hard won. The tragedy of thalidomide had a major influence on professional and public awareness, but continued vigilance is essential if further similar events are to be avoided. Current standards for testing of potential therapeutic agents for developmental toxicity has prevented any repetition of the thalidomide tragedy, and there have been no reported episodes of new unrecognized teratogens released into routine therapeutic use for more than two decades. Although the deleterious effects of some agents may appear idiosyncratic, the recognition and understanding of certain principles regarding the

harmful effects of drugs in general serve to guard against complacency. We now recognize that agents that bind to steroid hormone receptors, the aryl hydrocarbon receptor, or retinoid receptors are potential developmental toxins with likely teratogenic effects.

There is no effective maternal–fetal barrier against drugs ingested by pregnant women. Although for some substances the transplacental dispersion is concentration dependent, that is, dependent on the maternal dose ingested, it must be remembered that the placenta is a dynamic organ capable of facilitated and active transport by carrier molecules that may well increase placental transfer of a given substance to a greater extent than simple diffusion would permit. Thus it is possible that a drug or other molecule can achieve a higher concentration in the placenta and fetus than would normally be determined by the maternal serum concentration.

The harmful effects of drugs are substantially determined by the stage of development of the conceptus at the time of exposure. Thus developmental toxicity results from exposure in the embryonic period during which there is major organogenesis. This critical period extends from fertilization until approximately 60 days postconception, and the pattern of abnormality reflects the phase of organogenesis during the time of exposure. In the fetal period, that is 60 days postfertilization until birth, drugs may exert their deleterious influence by changes in the growth and functional development of organs. Drugs given late in pregnancy or during labor may also cause problems in the progress of labor or in the neonate postpartum. It should also be remembered that certain classes of drugs have long half-lives and can be teratogenic for months after the cessation of maternal therapy, for example, retinoic acid analogues.

Maternal ingestion of drugs that may affect the fetus can occur in the following circumstances:

1. Inadvertently, without the mother realizing she is pregnant
2. Taken in diagnosed pregnancy without consideration or knowledge of the risks involved
3. Therapeutic administration in the knowledge of pregnancy in the first trimester
4. Therapeutic administration in the knowledge of pregnancy in the second and third trimesters
5. Maternal administration of drugs intended to have a therapeutic effect on the fetus
6. Maternal therapies during labor
7. Maternal treatment postpartum in breast-feeding mothers

It has been calculated that approximately one third of all pregnancy women receive at least one course of drug therapy during pregnancy (Rubin et al. 1986). This apparently high rate, given the widespread understanding of the risks of drug ingestion in pregnancy, is a gross understatement of the true incidence of fetal exposure in the first trimester to pharmacological agents as self-treatment by proprietary over-the-counter (OTC) medications, and continuation of prescribed therapy is frequent prior to the mother or her medical advisers knowing she is pregnant. This may be particularly critical given the fact that exposure is occurring during the phase of organogenesis, which is the period of greatest risk to the embryo.

As it is not possible to conduct clinical trials of the effects of drugs in humans in early pregnancy, we rely on the results of anecdotal occurrence or therapeutic disasters to identify teratogenic agents, and only a small number of drugs are definitely regarded as known teratogens if administered in the first trimester of pregnancy. It should also be noted that teratogenic effects may be dose dependent or may require the coadministration of other agents or synergistic influences if serious sequelae are to ensue. An additional complication in assessing the teratogenic effect of any agent is the background rate of congenital malformation in the community as a whole, some of which may be teratogenic in its own right, which is in the order of 1% to 2% of all pregnancies. An example of this difficulty is the thalidomide experience, in which it is now clear that some cases of limb reduction defect were in fact Roberts syndrome and not the result of thalidomide exposure in the mother. This has become apparent when children of apparent thalidomide victims are born with identical patterns of limb deficiency. A significant proportion, perhaps 10%, of congenital abnormalities result from environmental influences

including preexisting maternal conditions, infective agents, mechanical disruptions, and chemicals, while in the majority of instances the etiology is unknown (Brent and Beckman 1992).

Also, we are continually exposed to numerous chemicals in the environment for which the teratogenic potential is largely unknown. It has been estimated that only approximately 5% of the 60,000 or more chemicals in commercial use have been assessed for their teratogenic potential. In the future, sophisticated structural analyses of chemicals may provide a means of predicting teratogenic potential and permitting a rapid assessment of risk for any given agent (McLachlan 1993; Ghanooni et al. 1997).

Only a few representative examples are described here, and the reader is referred to other sources for a general review and more detailed information (Rubin 1995, 1998).

Over-the-Counter Medications

Recent studies have shown that most pregnant women use OTC medications at some stage in their pregnancy. In many instances this use is in the critical developmental stages of the first trimester. Werler et al. (2005) reported that in the United States 65% of women had used acetaminophen, 15% had used ibuprofen, and 4% had used other drugs such as pseudoephedrine, aspirin, and naproxen during pregnancy. This rate of consumption exposes a huge population of developing babies to a vast array of agents. With such large numbers even a small toxic effect will give rise to a clinically important and avoidable rate of potentially deleterious results. Pain medication when taken in the first two gestational months of pregnancy is reported as strongly associated with stillbirths due to congenital anomalies and to be positively associated with all stillbirths (Pastore et al. 2000). Implicit in these findings is a potential explanation for a number of unexplained congenital anomalies and stillbirths, and it is clear that more must be done to monitor the use of OTCs in pregnancy if these risks to pregnancy are to be removed (Mitchell 2003).

Teratogenic Drugs

The serious effects of thalidomide on the fetus are well known (Leck and Miller 1962; Lenz 1962). Folic acid antagonists utilized as cytotoxic agents in cancer chemotherapy are also known to have serious effects on the developing embryo (Thiersch 1952; Milunsky et al. 1968). Of more immediate clinical import are commonly used agents that are proven teratogens. Examples of these include phenytoin, warfarin, retinoids, carbamazepine, lithium, sodium valproate, and danazol.

The teratogenic effects of anticonvulsant drugs was first described in relation to phenytoin by Meadow (1968). It is probable that other related compounds may have potentially harmful effects, and it has been suggested that there may be a potentiation of phenytoin effects with cotreatment with barbiturates. Children exposed to phenytoin present with a variety of malformations including dysmorphic facies, digital hypoplasia, nail hypoplasia, growth deficiency, and mental deficiency. More serious structural defects of organs such as the heart are also occasionally identified (Speidel and Meadow 1972; Monson et al. 1973; Hill et al. 1974). Of particular interest in relation to the effects of phenytoin is the apparent variation in the susceptibility of a fetus. The risk of a fetus exposed to phenytoin developing the full spectrum of effects is approximately 10%, with perhaps a third of fetuses having lesser abnormalities. Numerous studies now suggest that the fetal susceptibility depends on the fetal genotype, with inherited defects in phenytoin arene oxide detoxification contributing to the increased sensitivity to the drug (Buehler 1990; Finnell and Chernoff 1984; Strickler et al. 1985).

Warfarin embryopathy was first recognized in 1975, although previous case reports had described similar pathology in the babies of mothers with valve prostheses receiving anticoagulation, and is now well characterized (Becker et al. 1975; Pauli and Hall 1979; Ginsberg and Barron 1994).

Despite the condition being well recognized, new cases still occur (Frewin and Chisholm 1998; Wellesley et al. 1998). Approximately one third of exposed fetuses are born with the classical features of nasal hypoplasia, depressed nasal bridge, and stippled calcification of the epiphyses. A significant proportion also has mental retardation, and a variety of other abnormalities are recognized. The critical period of exposure appears to be between 6 and 9 weeks, but there is debate as

to the additional risks from exposure in the second and third trimesters, with reports of central nervous system abnormalities (Hall et al. 1980).

Retinoic acid embryopathy was first reported by Rosa (1983), and subsequently the spectrum of structural defects in prenatally exposed children has been described (Lammer et al. 1985). Retinoids are potent teratogens and give rise to craniofacial, cardiovascular, and central nervous system abnormalities. A particularly important feature of retinoic acid embryopathy is the long-term teratogenic potential of some retinoic acid analogues used therapeutically, particularly for the management of skin disease, for example, etretinate. The British National Formulary warns that some analogues may be teratogenic more than 12 months after the cessation of therapy.

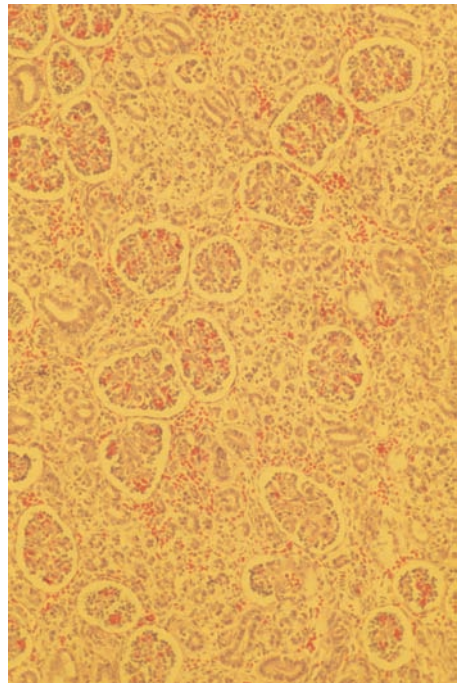

Figure 17.1. Renal tubular dysplasia secondary to fetal angiotensin-converting enzyme (ACE) inhibitor exposure. The proximal tubules have an immature morphology and the glomeruli are crowded.

Nonteratogenic Drug Effects

Drugs administered to mothers beyond the period of organogenesis can disrupt structural and functional growth and development of organs. Examples include the angiotensin-converting enzyme (ACE) inhibitors, sex hormones, antithyroid drugs, and beta-blockers.

Angiotensin-converting enzyme inhibitors such as captopril, are associated with fetal renal abnormalities including proximal renal tubular dysgenesis (Fig. 17.1) giving rise to neonatal renal failure (Cunniff et al. 1990; Barr and Cohen 1991). Intrauterine growth restriction and skull ossification defects are also frequently present. An increased incidence of intrauterine death, stillbirth, and perinatal death resulting from oligohydramnios and related abnormalities has also been described in the fetuses of mothers receiving ACE inhibitors.

Diethylstilbestrol was identified as having a transplacental carcinogenic affect in females. The majority of female children of mothers who received this drug in pregnancy developed vaginal adenosis, and a very much smaller proportion are at risk of subsequent development of adenocarcinoma (O'Brien et al. 1979). Male fetuses of exposed mothers developed genital anomalies.

Oral contraceptives, frequently taken in the first trimester of pregnancy, do not appear to be associated with a risk to the development of the fetus (Raman-Wilms et al. 1995).

The administration of antithyroid drugs can produce thyroid enlargement in the fetus (Fig. 17.2). These drugs readily cross the placenta and are thought to act by suppression of thyroxine production by the fetus with subsequent enhanced thyroid-stimulating hormone (TSH) secretion from the pituitary gland (Low et al. 1978; Mazzaferri 1997). The use of beta-blockers in the treatment of essential hypertension in pregnancy is associated with an increased risk of intrauterine growth restriction (Butters et al. 1990).

There are relatively few instances where maternal drug therapy need inhibit breast-feeding. Most drugs are secreted in the breast milk, but the dose ingested by the baby is usually insufficient to cause deleterious consequences. Atkinson et al. (1988) provides practical guidelines on the common drugs that pass into breast milk in significant quantities and make recommendations as to breast-feeding or drug treatment to be avoided

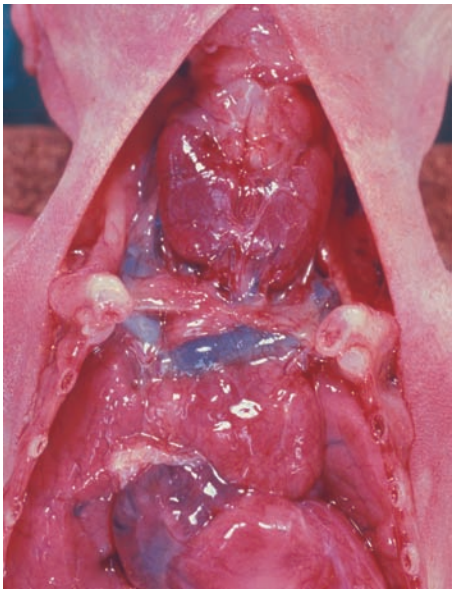

Figure 17.2. Thyroid enlargement in a fetus at 20 weeks' gestation exposed to carbimazole.

if breast-feeding is intended. Among the drugs that should be avoided in these circumstances are amiodarone, aspirin, barbiturates, benzodiazepines, and carbimazole. Cytotoxic agents are highly toxic, and breast-feeding is contraindicated for mothers on these therapies.

The potential for synergistic effects between drugs that are not thought to be teratogenic and other environmental influences should be considered. Hyperthermia is associated with the development of a variety of birth defects (Edwards et al. 1995). Animal experiments have identified potentiation of teratogenic effects of hyperthermia by aspirin in nonteratogenic doses (Tiboni et al. 1998). The effect is thought to be due to suppression of prostaglandin E, which is cytoprotective as a result of its induction of heat shock proteins.

Deleterious effects of intrauterine exposure to therapeutic agents need not be confined to structurally identifiable abnormalities. Recent work has raised the issue of more subtle effects that may manifest themselves in terms of organ function or effects on intellectual development of exposed individuals. Antenatal glucocorticoid therapy has reduced the rate of complications seen in preterm deliveries. Glucocorticoids have important effects on brain development and in animal studies can be shown to modify the structure and functioning of the brain. Recent work has suggested that the limbic system (specifically the hippocampus) and the hypothalamic-pituitary-adrenal axis are particularly sensitive to steroid exposure in utero with resultant alteration in behavior and learning performance. This complex and fascinating area is well reviewed by Matthews (2000). There is also increasing interest in the impact of prenatal glucocorticoid therapy on cardiovascular disease later in life (Dodic et al. 1999).

Not all harmful drug effects need necessarily be teratogenic or act directly on the fetus. Antibiotic prophylaxis for group B streptococcal infection is widely utilized particularly in the U.S. The recommended treatment protocols include the use of penicillin G or ampicillin. It has been shown that the antepartum use of ampicillin in this context appears to result in an increased incidence of early onset neonatal sepsis with non–group B streptococcal organisms that are resistant to ampicillin (Towers et al. 1998). This study also highlights the increased frequency of antibiotic utilization in pregnancy from a level of less than 10% in 1991 to 16.9% in 1996. The implications for antibiotic resistance and subsequent difficulties in neonatal care are obvious.

Drugs in Labor and Effects on the Fetus

Obstetric analgesia and anesthesia have the potential to affect the progress of labor, the fetus in utero, and the neonate after delivery.

The use of epidural anesthesia can have significant deleterious effects on the progress of labor. There is a decrease in uterine performance with an increased need for oxytocin augmentation, prolongation of the first and second stages of labor, and increased risk of operative delivery (cesarean section) (Thorp et al. 1993; Newton et al. 1995; Alexander et al. 1998). Both anesthetic gases and analgesic agents such as opiates pass readily across the placenta and into the fetus. These agents can cause respiratory depression, which may complicate the early neonatal period.

This complex and important clinical area is reviewed by Glosstein (1992). The reader is strongly encouraged to study this review article.

Complications of the Intrapartum Period

The pattern of complications that arise in relation to labor and delivery are the result of the interaction of maternal factors, the intrauterine well-being of the fetus and its position, and the decisions made by medical and nursing staff as to the manner of delivery. In this litigious era, it cannot be overemphasized that "birth injury" and related defects are as often the result of the fetal condition as they are the consequence of apparent errors of judgment on the part of medical and nursing staff supervising and managing the delivery. Therefore, pathologists should proceed with caution in attributing apparent traumatic abnormalities, particularly related to the head and intracranial lesions, solely to the attendants at a delivery.

It is the case that some facets of intrapartum asphyxia can be due to or accentuated by clinical decision making, but frequently asphyxiated babies are in poor condition as a result of prepartum intrauterine pathology affecting the placenta or have congenital defects that impair their capacity to withstand the normal rigors of labor. The complexities of this area are reviewed by Wigglesworth (1991).

Serious birth injuries do occur, however, and many of these are wholly traumatic in nature. The breech presentation is most likely to be associated with traumatic lesions. O'Mahony et al. (2005) reviewed singleton delivery intrapartum-related deaths in which traumatic cranial or cervical spine injury or difficult delivery was a significant feature. They found that the vast majority of cases meeting the criteria for inclusion in the study presented with fetal compromise prior to delivery. Where cranial and traumatic injury was seen, it was typically associated with a difficult instrumental delivery together with ill-judged persistence with attempts at vaginal delivery. Birth injury is discussed further in Chapter 13.

Elective cesarean section delivery is associated with a number of initial problems in the neonate. In the emergency situation the underlying pathology requiring urgent delivery by this route usually supersedes those abnormalities that result from cesarean section alone and that are manifest in babies born electively by this route and particularly those born prematurely.

Extracranial Hemorrhage

Edema and bleeding into the soft tissues of the scalp and extracranial tissues is not uncommon and most usually is of little clinical consequence.

Caput succedaneum is the accumulation of fluid and blood in the skin and superficial soft tissues of the scalp and usually affects the presenting part of the head over the vertex. It is thought to develop as the cervical canal compresses the skull during the passage of the head through the birth canal. This swelling usually subsides in a few days.

Chinon is a somewhat similar lesion resulting from the application of a Ventouse extractor with soft tissue edema underlying the area held by the extractor cap. In this instance the edema and hemorrhage is more tightly localized than with a caput.

Subaponeurotic or subgaleal hemorrhage originates deep to the epicranial aponeurosis, and substantial hemorrhage can accumulate in this layer and be associated with serious clinical consequences including hypovolemic shock (see Fig. 13.20 in Chapter 13).

Cephalhematoma is hemorrhage underlying the periosteum over the surface of the skull bones. This is usually a lesion limited by the boundaries of the individual skull bone plates and thus the volume of hemorrhage is usually much less than that seen in subaponeurotic hemorrhages. Simple linear fractures of the parietal bone are not infrequent in instances of cephalhematoma (Zelson et al. 1974) (see Fig. 13.21 in Chapter 13). Bofill et al. (1997) reported the development of cephalhematoma in 37 of 322 cases of delivery employing the vacuum extractor.

All of these extracranial fluid accumulations and hemorrhages have been associated with the use of the Ventouse vacuum extractor, particularly in instances of multiple applications as a result of technical failures in the procedure (Govaert et al. 1992; Benaron 1993; Florentino-Pineda et al. 1994).

Extradural hemorrhage is also often associated with skull fracture, but is usually of minor severity

and is located between the periosteum and the inner surface of the skull bones.

Skull Fractures

Fracture of the skull is most usually associated with forceps delivery but can also be seen as a result of pressure of the skull against the prominences of the maternal pelvis. Skull fracture has also been reported as a consequence of use of the Ventouse vacuum extractor (Hickey and McKenna 1996). Minor depressed skull fractures, most typically of the parietal bone, are usually of little clinical import. Similarly linear fractures involving only one skull bone usually do not lead to significant clinical sequelae. It is likely, therefore, that the frequency of skull fracture is higher than the reported incidents. Dupuis et al. (2005) reported that in a series of 68 cases of neonatally diagnosed depressed skull fracture managed in their unit, no fewer than 18 cases were of a "spontaneous" etiology, that is, not associated with instrumental delivery or use of the vacuum extractor.

More significant and more typical of a true traumatic birth injury is a multiradiate fracture of the skull bones, most typically affecting the parietal bones and frequently bilateral. These injuries are associated with significant intracranial hemorrhage as a result of tearing of subdural veins and of the venous sinuses. Serious intracranial injury is more likely to be associated with instrumental delivery (Dupuis et al. 2005).

In cases where a traumatic delivery results in formation of a leptomeningeal cyst, an associated fracture may grow in size in the neonatal period. A case has also been reported of expanding fontanelle secondary to delivery trauma with leptomeningeal cyst formation following use of the Ventouse extractor (Huisman et al. 1999).

Occipital Osteodiastasis

Wigglesworth and Husemeyer (1977) describe a serious fracture of the occipital bone resulting from disruption of the relationship between the squamous and lateral parts of the occipital bone, which are joined by cartilage and do not fuse until the second year of life. Pressure on the suboccipital region during delivery causes inward displacement of the squamous portion of the bone with resultant tearing of the underlying venous sinuses and subsequent hemorrhage often associated with direct injury to the cerebellum. In recent times this has not been a frequently reported pathology, although it is more likely to occur in vaginal breech delivery. Minor forms of this traumatic lesion can easily be missed unless specifically excluded by direct and careful inspection.

Subdural Hemorrhage

Subdural hemorrhage results from tearing of the bridging veins in the subdural space but can also follow tentorial and venous sinus hemorrhage resulting from precipitant or traumatic delivery. Subdural hemorrhage has also been described following the use of vacuum extraction (Hall 1992; Castillo and Fordham 1995).

Although many of these hemorrhages appear to be related to instrumental delivery, and in particular to the use of the vacuum extractor, it should not be forgotten that these lesions have also been described as arising in utero and not related to the delivery process.

Petrikovsky et al. (1998) reported seven cases of cephalhematoma and caput succedaneum not related to labor. Gunn and Becroft (1984) reported subdural hemorrhage arising in utero and identified in stillborn babies, and Demir et al. (1989) reported antenatal subdural hemorrhage that resulted in intrauterine death.

Extracranial Injuries

A large variety of additional injuries are reported related to birth. These include fractures, hemorrhage into soft tissues and related to major organs and injuries to the spinal cord and nerves. The risk factors and other morbidities associated with the development of these injuries includes birth weight greater than 4 kg, prolonged second stage of labor, use of epidural anesthesia and oxytocin, forceps delivery, shoulder dystocia, and fetal compromise as evidenced by meconium passage in labor and low Apgar scores (Perlow et al. 1996; Gherman et al. 1998; Beall and Ross 2001).

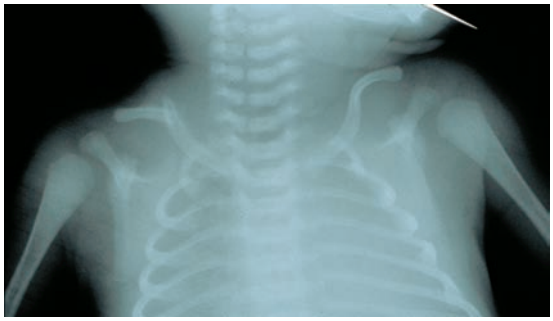

Figure 17.3. Birth injury healing mid-clavicular fracture at 11 days of age.

Fractures

Clavicular fractures are seen particularly in difficult deliveries of large infants or in cases of shoulder dystocia (Fig. 17.3). They are not uncommon in breech presentations. Published reports give variable incidence rates for this complication in the range 0.5% to 1.65% of deliveries (Perlow et al. 1996; Kaplan et al. 1998; Cohen and Otto 1980; Beall and Ross 2001).

Diagonal fractures of the middle third of the long bones, most frequently the femur and humerus, are well recognized (see Fig. 13.30 in Chapter 13). They are seen with normal deliveries but also more commonly in instances of breech presentation. In my experience fractures of the vertebrae are extremely rare, although again they are seen with breech delivery.

Visceral Injuries

Hemorrhage related to intraabdominal organs such as the liver, spleen, and kidney is not infrequent. The commonest pattern of hemorrhage is a subcapsular hematoma of the liver. These lesions are also seen in stillborn fetuses and in fetuses aborted for chromosomal abnormality or congenital malformation. Capsular rupture of the spleen is less common but can give rise to hemoperitoneum. Traumatic renal hemorrhage is extremely rare.

Injuries to the Spinal Cord

Spinal cord injuries are more likely to occur during breech delivery and have become less frequent with the increasing use of cesarean section in breech presentations. They are also seen, but much less frequently, in cephalic presentations, with injuries arising during delivery of the shoulder. The mechanism of injury is a combination of excessive longitudinal traction while the head is hyperextended and possibly ischemic damage related to either stretching with spasm or occlusion of the vertebral arteries (Yates 1959). I have not seen or recognized a case of gross spinal cord injury at postmortem examination in the last 10 years.

Peripheral Nerve Injuries

Injuries to the brachial plexus are probably the most common peripheral nerve injuries. An Erb's palsy results when the fifth and sixth cervical nerves are damaged, and Klumpke's paralysis results when the seventh and eighth cervical and first thoracic nerves are injured. In Klumpke's paralysis there is also a Horner syndrome as a result of the damage to the first thoracic nerve. Occasionally phrenic nerve palsy occurs on the same side as the brachial nerve injury with resultant diaphragmatic paralysis and respiratory embarrassment.

Perlow and colleagues (1996) report an incidence of facial nerve injury of 0.6 per 1000 live births and brachial plexus injury of 0.9 per 1000 live births.

These peripheral nerve injuries are frequently but not exclusively associated with shoulder dystocia, where the shoulder impacts against the symphysis pubis or the sacral promontory during delivery. The fetal manipulation techniques required for the delivery of a case of shoulder dystocia are not associated with an increased incidence of nerve injuries or fractures (Gherman et al. 1998). The main clinical risk factors are a large baby (thus the infants of diabetic mothers are at risk) and precipitant delivery with failure of truncal rotation and persisting anteroposterior (AP) alignment of the shoulders. However, the majority of cases occur in babies who are not overtly large, and it is therefore not necessarily possible to predict in advance the risk for an individual labor and baby (Levine et al. 1984; Acker et al. 1985; Sjöberg et al. 1988; Omu et al. 1995).

Complications Related to Cesarean Section Delivery

Babies born following cesarean section not only are at risk from the underlying pathological process necessitating this mode of delivery but also develop complications that result from the loss of the benefits of a vaginal delivery.

The vast majority of cesarean sections are performed for sound clinical reasons in the maternal or fetal interest. However, a not insignificant number appear to result for perhaps less clinically rigorous reasons. One study reported that 19.8% of 3150 elective cesarean sections were cases where a trial of vaginal delivery was considered appropriate but the mother requested an operative delivery (Wilkinson et al. 1998).

The incidence of respiratory distress syndrome and of transient tachypnea of the newborn is increased in babies born by the cesarean route (Parilla et al. 1993). Cesarean section delivery has been identified as an independent risk factor for the development of respiratory distress syndrome (Gerten et al. 2005). The etiology appears to be the retention of a relative excess of fluid within the lungs at the time of delivery. Normal vaginal delivery is associated with an adrenaline surge, which leads to a reduction in lung fluid volume (Walters and Olver 1978; Faxelius et al. 1983). In addition, there is increased synthesis of surfactant. Passage through the birth canal imparts a strong external compressive force on the thorax and aids the displacement of fluid from the lungs (Saunders and Milner 1978; Vyas et al. 1986). The loss of these physiological processes is associated with retention of excess liquor, reduced lung vital capacity (Segal and Chu 1963; Chiswick and Milner 1976), and lower mean thoracic gas volume (Milner et al. 1978).

Prenatal Diagnosis

The scope of prenatal diagnosis has expanded dramatically over the last two decades. It is now a routine part of obstetric care, and this development has been facilitated by improvements in ultrasound scanning and the advances in cytogenetics and molecular techniques. In excess of 600 conditions have been diagnosed prenatally, and the list continues to grow. Many of these conditions require the application of invasive techniques to sample the liquor around the baby, chorionic villi, or samples from the fetus directly. Any interference with the integrity of the uterus and gestational sac carries some risk for an individual pregnancy, and the monitoring of pregnancy losses that follow prenatal diagnostic procedures is an important part of the pathologist's role.

There is a large literature regarding the safety of the various invasive procedures employed in antenatal diagnosis. Both amniocentesis and chorionic villus sampling (CVS) have their proponents, but in general it appears that midtrimester amniocentesis is the safest procedure, while CVS and early amniocentesis have a slightly higher incidence of subsequent pregnancy loss (Alfirevic 2000; Alfirevic et al. 2000; Jauniaux et al. 2000).

Ultrasonography

Modern ultrasound machines have enormously increased the potential for prenatal intervention and diagnosis. Although the use of ultrasound is routine in obstetric practice, detailed scanning is operator dependent and ultrasound diagnoses are not infallible. Some anomalies can be identified with a very high success rate, for example, neural tube defects, but others, such as cardiac defects, are much more difficult to identify and diagnose.

There is no evidence that the use of ultrasound at diagnostic intensities has any deleterious effect on the fetus or the mother. However, with the development of new instruments with higher acoustic energies there is no room for complacency. Caution in the use of pulse and Doppler equipment in studies in the first trimester has been advised (European Federation of Societies for Ultrasound in Medicine and Biology 1991).

For the vast majority of instances of ultrasound use in pregnancy, the real risk would appear to be related to the skill of the operator and resultant misdiagnoses rather than the dangers of the standard equipment (Merritt et al. 1992).

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is now a routine antepartum and neonatal diagnostic tool,

particularly in instances of complex congenital malformation. It is of particular value in the assessment of lung size in cases of congenital diaphragmatic hernia, central nervous system abnormalities including hydrocephalus, and some cardiac malformations. There is no evidence that MRI scanning has any deleterious effect on the fetus or the progress of a pregnancy.

Amniocentesis

Amniocentesis is the most commonly used diagnostic intervention in pregnancy. It is usually performed at between 16 and 18 weeks' gestation when there is adequate liquor. Used in conjunction with real-time diagnostic ultrasound, it is a safe technique. Samples are usually taken by a transabdominal needle puncture using a narrow gauge needle of between 18G and 22G. The 22G needle is preferred as it has a lower rate of complications.

Certain complications are inherent in this invasive technique. Infection is a potential hazard but should be extremely uncommon with adequate aseptic technique. Secondary infection may lead to intrauterine fetal demise or spontaneous abortion secondary to intraamniotic infection and chorioamnionitis. In addition, it is known that fetal exposure to intraamniotic inflammation is strongly associated with the development of cerebral palsy in survivors (Yoon et al. 2000). In the case of women who are rhesus negative, it is necessary to provide anti-D treatment in order to prevent rhesus isoimmunization. In assessing the potential complications of amniocentesis, it is important to differentiate between midtrimester and early (9 to 14 weeks' gestation) amniocentesis as the range of complications is variable.

Midtrimester amniocentesis is associated with a significant increase in spontaneous and induced preterm delivery for which the etiology remains unclear (Medda et al. 2003). The principal risk of amniocentesis is the precipitation of spontaneous abortion and fetal loss. Early collaborative studies in the U.S. (NICHD 1976), Canada (Simpson et al. 1976), and Europe (Galjaard 1976) showed no increase in risk of spontaneous abortion as compared to the natural level for a given gestation. However, the Medical Research Council (MRC) collaborative study (MRC 1978) showed an increased fetal loss of 1% to 1.5% over controls, and another large randomized study (Tabor et al. 1986) cites an excess pregnancy loss rate of 1%. This lower figure probably reflects the use of high-quality ultrasound imaging and the improved training and expertise of the operators involved. The spontaneous abortions following amniocentesis probably result from the direct injury to the gestational sac. The MRC trial (1991) found that most abortions occurred in the week following the procedure. Previous studies had demonstrated that heavy blood-staining of the amniotic fluid sample or any reduction in fetal movement or fetal bradycardia following the procedure were predictive features. The NICHD study (1976) identified the use of the larger diameter 18G needle as being associated with increased risk of complication. A recent major review of studies incorporating over 68,000 midtrimester amniocentesis procedures concluded that the procedure-related excess pregnancy loss rate was 0.6% (Seeds 2004).

Significant fetal injury following midtrimester amniocentesis is not common. Small cutaneous scars resulting from direct needle puncture are described but are seldom of significance. Internal injuries of the fetus have also been described following inadvertent trauma. These injuries include fatal hemorrhage (Rushton 1981); intraabdominal pathology in the form of ileal atresia and peritoneal adhesions (Therkensen and Rehder 1981); limb anomalies resulting from arterial injury, constrictions, amputations, and intrauterine fetal demise secondary to amniotic bands (Lamb 1975; Rehder 1978; Strauss et al. 2000); and disruptive brain injury (Squier et al. 2000).

There were earlier reports of postural deformities (congenital dislocation of the hip and severe talipes) in babies subjected to amniocentesis in the midtrimester (MRC 1978). Subsequent studies have not supported these observations.

More significant sequelae of midtrimester amniocentesis relate to potential impairment of lung development and maturation. This was first identified in the MRC study (1978), and subsequently Tabor et al. (1986) reported an increased risk of respiratory distress syndrome and neonatal pneumonia. It was suggested that the fetal problems resulted from removal of amniotic fluid

and possibly from chronic amniotic fluid leakage that had not been noted by the patient.

Early amniocentesis, at 9 to 14 weeks' gestation, is associated with increased risk to fetal development. Although the procedure is technically similar to midtrimester amniocentesis, the fluid volume around the fetus is much smaller and it can be more difficult to obtain a sample. The incidence of unsuccessful attempts may be as high as 20%. The deleterious effects of amniocentesis on lung and limb development are the most obvious sources of concern. Milner et al. (1992) demonstrated adverse effects on lung function in cases of second-trimester amniocentesis, and Thompson et al. (1992) demonstrated an association with a reduced neonatal functional pulmonary residual capacity. There is clear evidence that the incidence of talipes is greater in the children of women undergoing amniocentesis prior to 14 weeks' gestation (Alfirevic 2000; Nikkila et al. 2002).

The risk of pregnancy loss is higher in early amniocentesis than with midtrimester amniocentesis (Assel 1992; Hanson et al. 1992; Alfirevic 2000; Nikkila et al. 2002). Transabdominal CVS provides an alternative to early amniocentesis, and given the increased complication rate there needs to be a careful balance of risks between the two procedures in individual cases (MRC Working Party 1991; Neilson and Gosden 1991; Alfirevic 2000).

Chorionic Villus Sampling

The need for early diagnosis of karyotypic or metabolic disorders thus permitting technically safe and easy medical termination of pregnancy has driven the development of CVS. Samples can be obtained either by a transcervical or a transabdominal approach. The transabdominal approach has an advantage for some practitioners in that the technique is similar to that used for amniocentesis in which practitioners are well versed.

Transabdominal sampling is performed using either a free hand technique with a standard needle or using a double-needle system with a guide needle attached to the ultrasound probe. With the double-needle technique, a smaller gauge needle is utilized and more frequent insertions for adequate tissue sampling are usually required. The transcervical approach utilizes either a thin plastic catheter or needle aspiration guided by ultrasound probe.

The complication rate increases with the number of needle insertions with a significant rise if three or more attempts are made (World Health Organization 1986; Brambati et al. 1987; Wade and Young 1989). It is hardly surprising that there is a significant incidence of fetomaternal hemorrhage following CVS by either technique. This can lead to maternal rhesus sensitization in instances of incompatibility or to a worsening of maternal immunization in a preimmunized patient. Patients are therefore checked for the need to receive anti-D immunoglobulin.

Several large randomized trials have shown that both the transabdominal and transcervical techniques are equally safe in the hands of experienced practitioners (Brambati et al. 1991; Smidt-Jensen et al. 1991; Jackson et al. 1992). The range of complications of CVS are wide and, while most are fortunately of minor clinical significance, some in individual cases can be more serious, giving rise to fetal anomaly particularly in the case of early CVS.

Vaginal bleeding of variable degree is a most frequent early complication and is usually more marked and more frequent with the transcervical technique. There does not appear to be a relationship between the extent of this bleeding and fetal loss, however. The rate of fetal loss following CVS is reported as between 2.3% and 2.5% (Rhoads et al. 1989).

Firth and colleagues (1991) reported a cluster of limb reduction defects in babies of a series of women who underwent CVS before completing 9 weeks' gestation. Two subsequent studies identified similar pathologies (Brambati et al. 1992; Burton et al. 1989), and it was proposed that these limb abnormalities were the result of vascular disruption and hypoxic tissue damage related to the needle movements. In contrast, a number of very large scale reviews showed no difference in the incidence of limb reduction anomalies in patients who were exposed to CVS as compared to other pregnancies (Froster-Iskenius et al. 1989; Kuliev et al. 1993; Kuliev et al. 1996). However, the consensus of opinion is that a causal relationship between the risk of limb reduction defects and CVS exists and that the critical period is prior to 9 weeks' gestation. In expert hands using good

ultrasound visualization and care with the needle, the risk is extremely remote.

Several studies have compared the risk of CVS versus amniocentesis. The consensus view has been that although CVS is a more difficult sampling technique and therefore should be confined to specialist centers with expertise and adequate numbers of cases, there was no difference in safety and efficacy of these techniques when there are high levels of expertise available (Canadian Trial Group 1989; Rhoads et al. 1989; MRC 1991; Smidt-Jensen et al. 1991; Brambati and Tului 2005). However, a Cochrane Database Review (Alfirevic et al. 2000) of over 9000 cases of second-trimester amniocentesis and CVS concluded that pregnancy loss was more common after CVS (odds ratio 1.33) and that the increased risk of loss appeared to be procedure-related.

In contrast a Cochrane Database Review comparing early amniocentesis with transabdominal CVS showed that transabdominal CVS was associated with fewer instances of spontaneous miscarriage and a lower incidence of neonatal talipes than early amniocentesis. The patients in the CVS group had a higher rate of sampling failure and subsequently a higher rate of second diagnostic testing. Neonatal hemangiomas were more frequent in the CVS group (Alfirevic 2000).

A long-term follow-up of infants in pregnancies that had transcervical CVS or amniocentesis concluded that there was no difference in the incidence of congenital malformations, neonatal morbidity, pediatric morbidity, or functional disturbance between the two patient groups (Schaap et al. 2002).

Cordocentesis

Fetal blood sampling is now a well-established procedure that has applications in a number of clinical situations. The usual sampling site is the placental insertion of the umbilical cord, but other sites that can be employed include the fetal cord insertion, the fetal intrahepatic vein, and the fetal heart. Needle insertion (20G or 22G spinal needle) is under continuous ultrasound visualization. It is important that the fetal heart be observed throughout the procedure, as fetal bradycardia indicates fetal distress, and the site of needle insertion is observed during and after the procedure in order to assess hematoma formation in the cord root and the invariable blood leakage from the puncture site (Fig. 17.4). Sampling is more problematic before 18 weeks' gestation, and there is a higher rate of pregnancy loss in these early-gestation pregnancies (Orlandi et al. 1990). The specific indications are the provision of rapid and uncontaminated fetal karyotype, the investigation and management of rhesus hemolytic disease, and the investigation and management of hematological disorders including autoimmune idiopathic thrombocytopenia and hemoglobinopathies. Fetal intrauterine infection can also be investigated using fetal blood samples.

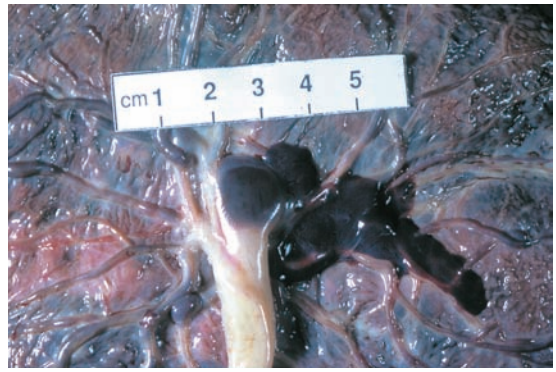

Figure 17.4. Small hematoma at the placental cord insertion following fetal blood sampling.

Many pregnancies where fetal blood sampling is done are, by definition, high risk. This complicates assessment of fetal loss related to the procedure alone. Loss rate estimates have been in the range of 1% to 2% (Daffos et al. 1985), but higher rates are reported in high-risk cases (Nicolaides 1988; Nicolini et al. 1990). In one major study the fetal loss rate for structurally normal fetuses was 1%, but this increased to 25% in a group of fetuses with nonimmune hydrops fetalis (Maxwell et al. 1991).

Fetal Tissue Biopsies

Several fetal tissues have been the subject of intrauterine biopsy for diagnostic purposes. Nicolini and Rodeck (1992) reported a series of 52 cases of intrauterine skin biopsy taken at 15 weeks' gestation and using 20-gauge forceps induced through an 18-gauge cannula. They found no

significant scarring resulting from these biopsies. Skin biopsy is typically taken for diagnosis of serious skin conditions such as epidermolysis bullosa. Liver biopsy is indicated in cases where there are enzyme deficiency disorders that are not yet amenable to diagnosis by DNA analysis. Fetal muscle biopsy is reserved for those cases of Duchenne muscular dystrophy that cannot be detected by DNA probes. Tru-Cut biopsy needles have been used to take the biopsies that have then been subjected to immunohistochemical analysis (Evans et al. 1991).

Fetoscopy and Fetal Surgery

Fetoscopic intrauterine interventions can be separated into two broad categories. The first is obstetric endoscopy, which includes surgical interventions on the placenta, umbilical cord, and fetal membranes, and the second is endoscopic fetal surgery. The current experience of these techniques is reviewed by Gratacos and Deprest (2000).

Obstetric Endoscopy

The most frequent obstetrically related intervention is neodymium:yttrium-aluminum-garnet (Nd:YAG) laser coagulation of placental vessels in cases of twin-to-twin transfusion syndrome. The incidence and outcomes of complications in 175 cases were reviewed by Yamamoto et al. (2005). The cases were treated prior to 26 weeks' gestation, and there were three abruptions and 12 miscarriages in their series. Premature rupture of membranes occurred subsequently in 28% of cases, some as late as 3 months after the laser therapy, and it is not clear whether there was a causal relationship in all cases. Given that twin-to-twin transfusion syndrome can complicate up to 15% of monochorionic pregnancies and presents with a mortality rate of 80% or more without intervention, the results of this study suggest that the technique is effective and relatively safe.

Closed Fetal Surgery

The most frequent form of closed interventions are those involving the placement of a double pigtail-ended fetal-amniotic shunt for the drainage of pathological fluid collections in the fetus. In these cases the decision to perform a drainage procedure is dependent on the exclusion of karyotypic anomaly and other serious fetal anomalies. Pleural effusions (Rodeck et al. 1988) and dilatations of the urinary tract resulting from obstruction at all levels from the pelviureteric junction to the posterior urethra are amenable to intrauterine drainage. For good prognosis cases, that is, those fulfilling specific pretreatment criteria (Cromblehome et al. 1990), the results of therapy are very good, with a fetal survival approaching 90% as compared to 70% in untreated cases. In poor prognosis cases that in untreated situations result in 100% fetal loss, the survival rate is on the order of 30%. Abdominal wall hernia has been reported as an uncommon complication of uterovesical amniotic shunt treatment for obstructive uropathy. The hernias were amenable to postnatal repair. In a report of three cases the authors noted that while the drainage of urine into the amniotic sac improved pulmonary development in all three patients, two of the three had renal failure requiring dialysis after birth (Gehring et al. 2000).

A potentially very significant advance has been the treatment of diaphragmatic hernia by selective plugging of the trachea resulting in the expansion of the obstructed lungs, which can displace herniated intestine into the abdomen. It should be cautioned, however, that the currently available evidence suggests that although there is lung enlargement following in utero tracheal occlusion, this appears to be due to abnormal dilatation of peripheral lung saccules with pooling of mucin. The lung remains structurally abnormal with low radial alveolar counts and abnormally large alveolae. The treatment did not prevent the development of lung pathology typically associated with pulmonary hypoplasia (Heerema et al. 2003).

It can be expected that closed fetal surgical procedures will increase dramatically in number and scope in the next 5 to 10 years as improved endoscopic techniques and the development of specific fetoscopic instruments together with better management of tocolysis become available (Sydorak and Albanese 2003).

A recognized hazard of techniques that breach the amniotic sac is rupture of the membranes with amniotic fluid leak or premature delivery. Most cases can be expected to seal spontaneously if

infection does not develop, but active interventions to plug leaks either with an amnio patch of platelets and cryoprecipitate or application of fibrin sealant has been successfully reported (Quintero et al. 1999; Young et al. 2000).

Open Fetal Surgery

Many of the more complex fetal anomalies that severely compromise the fetus to the point where extrauterine existence is called into question are as yet not amenable to repair by closed techniques. Because survival rates are so poor, these conditions have led to the development of open fetal surgical techniques. Urinary tract obstruction, diaphragmatic hernia (Harrison et al. 1990), congenital cystic adenomatoid formation, and sacrococcygeal teratoma have all been the subject of fetal surgery over the last 10 years. While there have been some successes, the effects on fetal outcome do not appear to be dramatically favorable (Kuller and Golbus 1992), and in fact open fetal surgery for repair of congenital diaphragmatic hernia has been virtually abandoned in North America. The subject is reviewed by Farmer (1998) and Cass (2005). The risk to the mother appears to be acceptable, and although preterm labor is the rule as a result of failure of tocolysis, there does not appear to be any increase in maternal compromise.

Complications of Neonatal Therapy

The diverse patterns of pathology that are seen in neonates results in part from the immaturity of these patients, both those born at term and premature neonates, and the unavoidable consequences of invasive and often highly aggressive therapeutic modalities invoked in their care. Many neonates who require active therapeutic intervention are extremely ill and represent very high risk therapeutic challenges to neonatologists. Any pathological lesions or complications that develop in these infants may be the result of instrumentation, procedures required for monitoring or the damaging effects of primary pathologies of prematurity, or pathologies resulting directly from therapeutic intervention. Superimposed on these pathological processes are developments in the genesis of lesions that become apparent only as critically ill neonates survive for prolonged periods before their ultimate, and often inevitable, demise. Thus pathological lesions are now seen that would not have been apparent to preceding generations of pathologists involved in perinatal and neonatal medicine.

The whole spectrum of pathological appearances that are seen in neonatal medicine varies as new therapeutic modalities are introduced and older treatments are abandoned. It is therefore incumbent on pathologists to pay particular attention to the patterns of therapy employed and to record with care and accuracy the abnormalities seen. Only in this way can potentially serious deleterious consequences of innovative treatments be identified at an early stage, thus avoiding unnecessary or unacceptable injury to patients.

It should not be forgotten, however, that standard and routine interventions can cause cosmetic or functionally deleterious lesions during neonatal intensive care. Skin damage is not infrequent, and, while usually trivial, a cautionary report (Cartlidge et al. 1990) identified 11 cases of significant skin injury with permanent sequelae in a study group of 100 neonatal intensive care survivors. The injuries were caused by chest drain insertion, extravasation of intravenous fluid, and skin stripping by adhesive tape.

Respiratory System

The most significant patterns of iatrogenic pathology in neonates relate to the need for ventilatory support in premature neonates or neonates who have other causes of respiratory distress and hypoxemia. Over the last several years the range of options available to neonatologists for maintenance of oxygenation has increased dramatically with the concomitant development of iatrogenic lesions. The majority of these techniques maintain the need for intubation of the proximal airways, but techniques of cardiopulmonary bypass have also been introduced into neonatal units.

Injuries Caused by Endotracheal Intubation

Cutaneous erythema and superficial ulceration around the nose and mouth are very common in

intubated neonates. They result both from the use of adhesive tape and from direct irritation of the poorly keratinized skin of premature infants, which withstands friction poorly. Nasal intubation by endotracheal tubes and nasogastric tubes can give rise to more serious pathology, and large-bore endotracheal tubes can cause significant damage to the nasal septum (Fig. 17.5).

Abnormalities of primary dentition have also been identified in infants intubated for prolonged periods and are thought to be the result of pressure effects of the endotracheal tube on the gingival margin (Fearne et al. 1985). Grooves and clefting of the palate have been described in patients with long-term endotracheal intubation. Fadavi and colleagues (1992) reported on a group of 52 prematurely born children who when examined between the ages of 2 and 5 years demonstrated significant palatal deformities and abnormalities of dentition. These are thought to arise from direct pressure effects of the tube. Alternative mechanisms have been suggested by Carillo (1995) and Hanson et al. (1976). In my experience these abnormalities are usually minor in modern practice, possibly reflecting the availability of a better range of endotracheal tube sizes made of less traumatizing materials. Pape et al. (1976) described deformity of the skull and associated cerebellar hemorrhage secondary to venous infarction in patients on whom face masks were secured by Velcro bands.

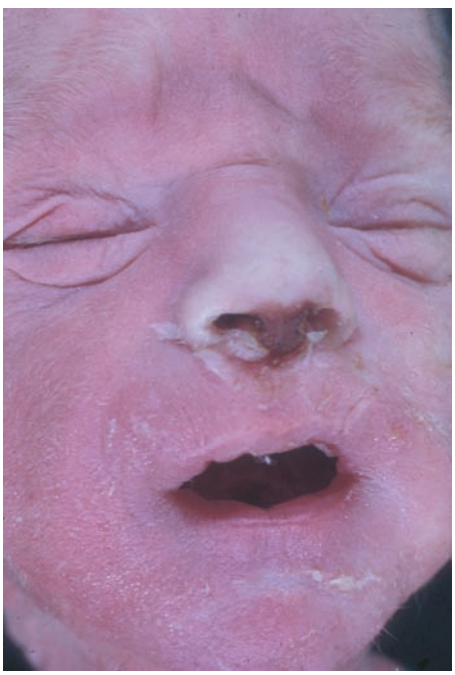

Figure 17.5. Ulceration of the nasal septum after endotracheal intubation.

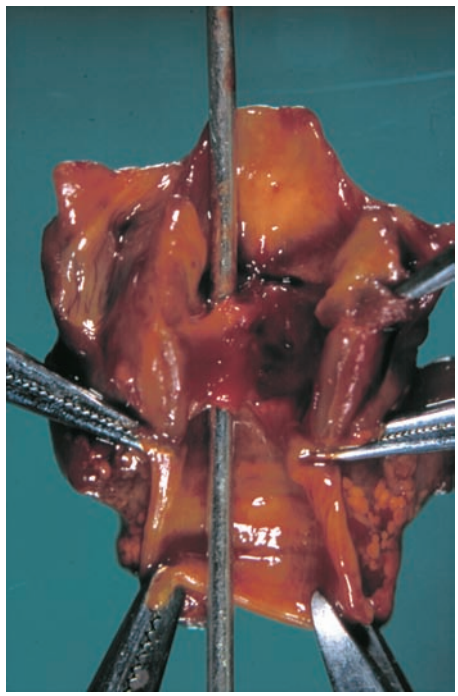

Figure 17.6. Laryngeal mucosal tear with false passage formation following difficult endotracheal intubation.

The physical process of intubation can damage the pharynx, esophagus, and upper airway structures, although fortunately these injuries, usually perforations or tears, are rare (Espinosa and Paredes 1974; Lynch et al. 1974; Reynolds and Taghizadeh 1974; Clarke et al. 1980; Holcomb and Templeton 1989) (Fig. 17.6). Sapin et al. (2000) reported the outcome in a series of 10 patients, five of whom were managed conservatively while the remainder required surgical interventions, and it was noted that the outcome was not always favorable principally as a result of concomitant pathology of prematurity.

Foci of ulceration of the larynx in the region of the vocal cords or subglottic region are frequently seen (Fig. 17.7). The majority of these lesions are superficial and heal without significant scarring or fibrosis after the removal of the endotracheal

tube. The lesions appear to be the result of direct pressure effects of the tube and its inflated cuff. More rarely the ulceration is deep and heals by fibrosis with narrowing of the airways following scar maturation and shrinkage (Liu et al. 1995). O'Neill (1984) estimated that laryngeal or tracheal stenosis occurred in 1.5% of cases at risk, and that intubation for periods of greater than 4 weeks' duration was a predisposing factor. Perichondritis and chondromalacia affecting the arytenoid and cricoid cartilages have been described as a sequel to prolonged intubation (Gould and Howard 1985).

Ulcerative foci in the tracheal mucosa are rarely seen and usually present as a vertical row of shallow ulcers on the anterior midline surface of the tracheal rings. These clearly result from direct contact with the endotracheal tube. The selection of a tube of an appropriate size should militate against this development. More common is squamous metaplasia of the anterior portion of the tracheal mucosa in those parts of the trachea in contact with the tube (Fig. 17.8). This metaplastic change in response to direct irritation may theoretically interfere with the mucociliary escalator and thus with mucus clearance from the proximal airways. This could predispose to infection and appears to be a lesion that persists for some considerable time after removal of the endotracheal tube. The repeated use of suction as part of the standard endotracheal toilet in neonatal intensive care can also result in tracheal and upper bronchial injury if the aspirating cannula is inserted too far distally. It is generally accepted that it is not necessary to aspirate the bronchi but merely to keep the tube itself clear. Mucociliary activity of the airways distal to the end of the tracheostomy tube keeps the proximal major airways clear without need for suction in otherwise uncomplicated situations. Subglottic mucous cysts have been described in patients with long-standing endotracheal intubation and may compromise airway patency after removal of the endotracheal tube (Downing et al. 1993).

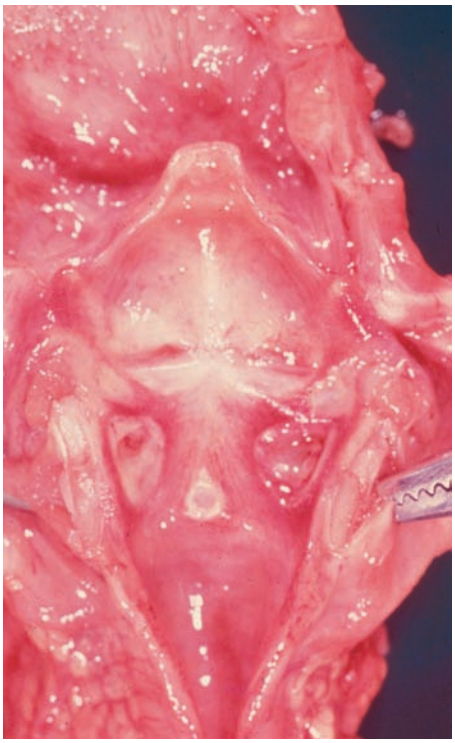

Figure 17.7. Larynx opened posteriorly. Ulceration is present in the midline anteriorly and on both sides below the vocal folds; intubation injury.

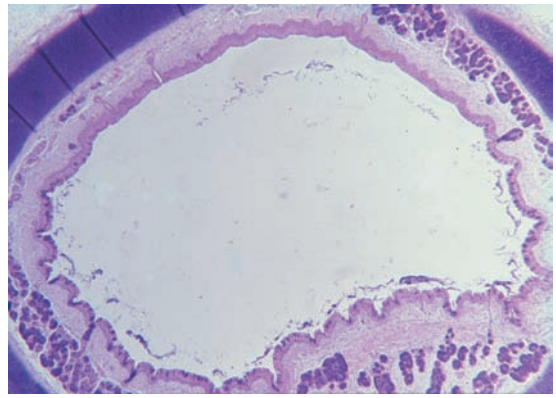

Figure 17.8. Cross-section of the trachea. Epithelial squamous metaplasia is present in the anterior half.

It is clear that endotracheal intubation can give rise to a number of pathological processes, but the appropriate selection of tube size, gentle handling without excessive vigor in aspiration of the tube, and the use of humidified ventilating gases greatly minimize the risk of these developments.

Patent Ductus Arteriosus and Fluid Overload

A patent ductus arteriosus is associated with a higher incidence of chronic lung disease in infants, and this is particularly the case with low birth

weight neonates (Ehrenkranz et al. 1978; Brown 1979). There is also a strong association with fluid overload and the development of chronic lung disease. Thus any failure to recognize or manage the development of pulmonary edema can be expected to increase the risk of chronic lung disease in a ventilated neonate.

Complications of Assisted Ventilation

Neonatal respiratory disease results from the interrelationship among maternal health, the presence or absence of prematurity, and the consequences of medical interventions. Prematurity is the most important etiological factor in the development of respiratory distress syndrome and results from factors linked to maternal health and obstetric care. The combination of prematurity and medical interventions result in other pathological consequences including pneumothorax, pulmonary interstitial emphysema, and chronic lung disease. The etiology and spectrum of iatrogenic injury is reviewed by Clark (1999).

Respiratory distress syndrome is the commonest cause of death in the neonatal period and the incidence in the United Kingdom has been estimated at 9.6 per 1000 live births per year (Field et al. 1987). This results in approximately 7000 babies per annum developing respiratory distress syndrome in the U.K. and reflects a very large therapeutic load on neonatal units. The incidence of respiratory distress syndrome is related to the number of premature deliveries. The maternal factors that are involved are predominantly beyond the iatrogenic area. However, modern obstetric practice and confidence in the therapeutic capabilities of the neonatal intensive care have led to a higher incidence in elective preterm delivery, with up to 30% of such deliveries being the result of deliberate therapeutic obstetric decisions (Rush et al. 1976, 1978; Berkowitz 1981).

Respiratory Distress Syndrome and Chronic Lung Disease

These topics are discussed in detail elsewhere in this book (see Chapter 20). Chronic lung disease includes the pathological disorder bronchopulmonary dysplasia (BPD), which was first described in 1967 by Northway et al. The pathology of chronic lung disease is very heterogeneous and involves abnormalities in the airways, blood vessels, and interstitial tissues (Van Lierde et al. 1991). The evolution of chronic lung disease and bronchopulmonary dysplasia are generally regarded as passing through a series of pathological stages, but it should be noted that these are not defined absolutes and that there is variability in progress of the pathology and in the distribution of injury within the lung. The early stages of acute lung injury involve direct damage to the alveolar epithelium including the type 2 cells, exudation, and formation of hyaline membranes. There is necrosis of the epithelium lining the distal bronchioles, and interstitial edema, congestion, and cell exudation are prominent. Organization of the intraalveolar and bronchiolar exudate may follow. In the final stages there is alveolar septal fibrosis with geometric distortion, metaplastic changes in the bronchial lining cells, and the potential for development of pulmonary hypertensive changes in peripheral arterioles.

The most important etiological association with chronic lung disease is respiratory distress syndrome, but also significant are oxygen toxicity, positive pressure ventilation, patent ductus arteriosus, and pulmonary air leak. Infection can also play an important contributory role in the evolution of the pathological processes.

Although the changes of bronchopulmonary dysplasia can be produced in animals exposed to high levels of oxygen only (Bonikos et al. 1976; De Lemos 1987), the practical reality is that the condition was not seen to any extent in neonates before the advent of assisted mechanical ventilation. It therefore represents an archetypal iatrogenic pathology.

Advances in neonatal intensive care and in particular the antenatal use of corticosteroids and postnatal surfactant therapy have modified the pattern of neonatal chronic lung disease such that the classical progression of bronchopulmonary dysplasia is seen infrequently. The histology of chronic lung disease of neonates now reflects more basic disorder of normal pulmonary development with deficiency of structural elements and excessive development of mesenchymal components. The subject is reviewed by Bland (2005).

Oxygen Toxicity

High oxygen concentrations in inspired or ventilated air have dramatic effects on the cells of the airways and lungs, most particularly the alveolar type 2 epithelial cells (Crapo et al. 1978). The evidence for the injurious effect of pure oxygen is clear, but what is much less certain is whether oxygen concentrations of 90% or less cause significant injury (Nash et al. 1967). Oxygen induces tissue injury by increasing formation of free radicals, which are highly reactive and react with membrane lipids and other intracellular constituents. Many of the antioxidant defense mechanisms of the neonate are immature, and the neonate is unable to respond by dramatically increasing antioxidant enzyme activity when challenged with hyperoxia (Frank and Sosenko 1987, 1991).

Positive Pressure Ventilation

There is considerable evidence in support of the view that intermittent positive pressure ventilation (IPPV) is the main etiological factor in BPD. The significance of positive pressure ventilation in the genesis of chronic lung disease and BPD was recognized by Barnes et al. (1969), and Tooley (1979) defined the relationship more clearly. Peak inspiratory pressures in the excess of 35 cm of water were highly associated with significant and serious BPD (Taghizadeh and Reynolds 1976). The full spectrum of pathology will develop with IPPV in the absence of hyperoxia.

Pulmonary Air Leak

Central to the process of ventilation is the requirement to deliver oxygenated gas to the air–blood interface in the lung periphery. This requires ventilating pressures sufficient to achieve alveolar expansion, and in situations of prematurity with surfactant deficiency this pressure must be maintained throughout the ventilatory cycle in order to prevent alveolar collapse with loss of capacity for gaseous exchange.

Although ventilation pressures are maintained at the lowest level commensurate with adequate oxygenation, the pressures are always such as to increase the risk of pulmonary air leakage. This becomes particularly likely if pulmonary compliance falls and ventilation pressures have to rise significantly. In my experience some degree of passage of air into the interstitial tissues of the lung is universal in ventilated neonates.

Pulmonary air leaks are secondary to alveolar distention, but the sites of tissue rupture are difficult to identify in babies who have been ventilated for prolonged periods or who have developed significant additional pathologies prior to their presentation for postmortem examination. Alveolar overdistention is particularly associated with high transpulmonary pressure swings, air trapping, and uneven alveolar ventilation. Air leaking from the gaseous spaces will track along preformed anatomical pathways particularly around bronchi, bronchioles, and perivascular tissues. The air may be localized to only one lobe, may extend into the mediastinum and soft tissues of the head and neck, or may rupture directly into the pleural cavity, giving rise to a pneumothorax. Extrathoracic extension of pneumomediastinum is well recognized.

Pneumothorax

While spontaneous pneumothorax is seen in up to 1% of babies at the time of birth (Steele et al. 1971), the vast majority of pneumothoraces are related to pulmonary pathology secondary either to prematurity or to other disorders requiring ventilation.

The instance of pneumothorax increases as the level of respiratory support increases (Madansky et al. 1979). The application of positive expiratory pressure in an effort to maintain alveolar distention in situations of surfactant deficiency is associated with increased incidence of pneumothoraces (Berg et al. 1975). High inflation pressures and mean airway pressures greater than 12 cm of water are associated with increased risk of pneumothorax (Oh and Stern 1977; Greenough et al. 1984; Tarnow-Mordi and Wilkinson 1985). Another well-recognized disorder that predisposes to pneumothorax is the development of active expiratory efforts by the infant during the positive pressure plateau of assisted ventilation, that is, “fighting the ventilator” (Greenough et al. 1983). The incidence of neonates fighting the ventilator can be increased by therapeutic protocols, and logically attention to ventilation rate and duration of ventilation time can militate against this

pathology, indicating that there is an iatrogenic component beyond the presence of abnormal pressures applied to the airways. Increasing the ventilation rate has a beneficial effect in lowering the rate of pneumothoraces and reduces the incidence of active expiration and fighting the ventilator (Field et al. 1985; Greenhough et al. 1986). The use of high-frequency jet oscillation has also demonstrated a significantly lower incidence of pulmonary air leak and pneumothoraces (high frequency oscillatory (HiFO) Study Group 1993).

Pulmonary Interstitial Emphysema

Interstitial air leak may be localized to one lobe of a lung but more commonly affects both lungs. In both instances the presence of pulmonary interstitial emphysema (PIE) can be recognized macroscopically by the presence of air blebs under the pleural surfaces of the lung (Fig. 17.9). Sectioning of the lung also reveals small cystic spaces in relation to interlobar septa and the larger interstitial tissue planes. Pulmonary interstitial emphysema is a potentially serious condition causing lung splinting with impaired ventilation and hypoxemia. Rarely the accumulation of gas around one lobe of the lung may be sufficient to cause compromise of the lung (Fig. 17.10) and even mediastinal shift giving rise to significant respiratory embarrassment. This has been treated by lobectomy (Drew et al. 1978), but it is more common to attempt management by variation of ventilatory care using high-frequency ventilation and withdrawal of positive end expiratory pressure. Other surgical therapeutic treatments have involved direct insertion of chest drains into the larger subpleural blebs (Roberton 1976) or the use of linear pleurotomies (Milligan et al. 1984). Zerella and Trump (1987) reported a series of PIE decompressions by thoracotomy with lysis of the individual blebs of air. Seventeen of the 31 patients treated survived the procedure, with mortality being more common in those neonates with poor clinical prognostic features.

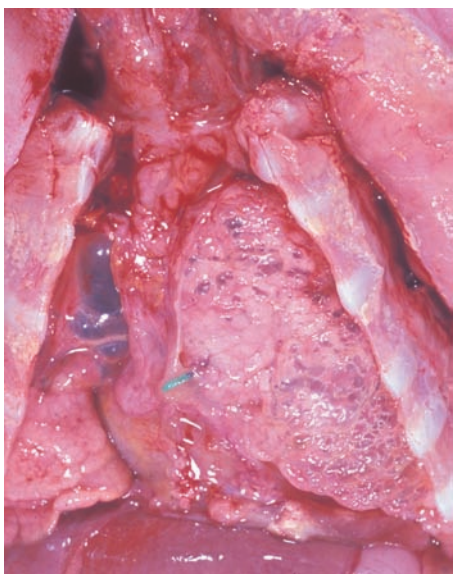

Figure 17.9. Interstitial emphysema involving both lobes of the left lung. Gas bubbles are visible through the pleura. There is gas accumulation at the right hilus.

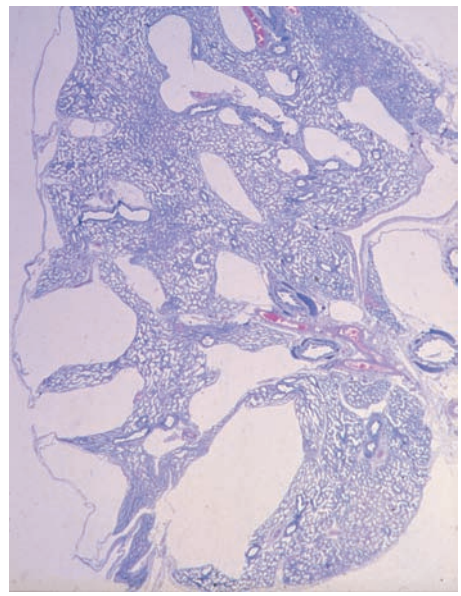

Figure 17.10. Interstitial emphysema. Large accumulations of gas distort the lung architecture.

Extrapulmonary Air Leakage

Pneumomediastinum is not uncommon in cases of respiratory distress syndrome (RDS) requiring ventilation. Postmature infants are at increased risk. In some instances air may track into the soft tissues of the neck, giving rise to subcutaneous emphysema. Most usually the patients are asymptomatic or have mild respiratory

signs. Pneumopericardium frequently occurs with pneumomediastinum, with air entry into the pericardial sac probably adjacent to the pericardial reflection near the ostia of the pulmonary veins. Pneumopericardium is rarely asymptomatic and usually causes cardiac embarrassment with tamponade-like symptoms (see Fig. 2.9 in Chapter 2). Pneumoperitoneum can arise as a result of air accumulation within the chest with dissection via the diaphragmatic foramina into the intraperitoneal space. It is more usually associated with the infants who already have pneumothorax or pneumomediastinum. Unless the abdomen is under tension, there is no need for active treatment.

Pulmonary Gas Embolism

This is a rare complication of positive pressure ventilation (Lee and Tanswell 1989). The embolism results from direct communication between the airways and small vascular channels (Bowen et al. 1973). The lesion is more likely in situations where there is laceration of lung tissue perhaps as a result of instrumentation (see below) that favors reversal of the intrabronchial pressure-pulmonary venous pressure gradient (Chiu et al. 1967). Pulmonary gas embolism is usually fatal.

Other Ventilator Injury

High-frequency jet ventilation in which aliquots of gas are fired into the airways via an endotracheal tube and cannula at a rate of 200 to 600 per minute has been associated with the development of necrotizing tracheobronchitis characterized by the development of tracheal, mucosal, and submucosal ischemic injury. Mucosal inflammation, erythema, and erosion are relatively minor patterns of injury, but more serious tracheal necrosis and resultant tracheal obstruction are reported (Fox et al. 1984; Boros et al. 1985; Mammel and Boros 1987). The lesions are not simply the result of direct impact of the gas jet as they have been reported when the tracheal wall was not in the line of the jet (Chan et al. 1988). Not dissimilar tracheal lesions have also been reported with other high-frequency ventilation systems. Inflammatory endobronchial polyps have been seen in children who have had a history of mechanical ventilation in the neonatal period (McShane et al. 2002). The authors suggest that these lesions result from airway trauma, but it is not clear as to whether this was the result of suction cannulation direct injury or pressure effects.

Special Techniques

Extracorporeal Membrane Oxygenation

Extracorporeal membrane oxygenation (ECMO) is a form of cardiopulmonary bypass that has been introduced into neonatal intensive care as a method of oxygenation that avoids the complications of barotrauma secondary to positive pressure ventilation (Bartlett et al. 1982).

There are two forms of ECMO. The first, veno-arterial (VA), involves the creation of a circuit with blood taken from the right jugular vein and returned via the right common carotid artery (Andrews et al. 1986), and the second, venovenous (VV), involves blood taken from the right jugular vein and returned usually through the femoral vein (Andrews et al. 1983; Klein et al. 1985). Venoarterial ECMO is more advantageous in that there is approximately 80% cardiopulmonary bypass, and thus there is a dramatic reduction in the level of respiratory support required. A disadvantage is that there is a potential for embolization of blood clot or air into the arterial circulation and in particular to the central nervous system. In addition, the ligation of the carotid artery or the attempted reconstruction of the carotid artery following decannulation can give rise to additional complications (Schumacher et al. 1988; Crombleholme et al. 1990). With VV ECMO there is no cardiopulmonary bypass, and the infant is dependent on good myocardial function. Femoral vein ligation after decannulation may give rise to obstruction of venous drainage to the limb and consequent edema.

In both forms of ECMO the venous blood is oxygenated outside of the body and returned by a pump after passing through a heat exchanger. The patient needs to be heparinized and sedated throughout the treatment.

The results of the U.K. trial of ECMO revealed the successful nature of the therapy, but also indicated the high morbidity and mortality that results from the underlying presenting primary pathologies (U.K. Collaborative ECMO Trial Group 1996),

as most infants eligible for ECMO are critically ill. Complications related to ECMO are not infrequent and, in a minority of cases, may be serious. It is important to note that pathology established prior to the commencement on ECMO, particularly that related to the lung and resulting from prematurity, hypoplasia, and barotrauma, will progress through the usual stages despite the cessation of ventilation while on VA ECMO (Pratt et al. 1979) and will manifest itself in survivors later in childhood in the form of hyperinflation, airways obstruction, and lower oxygen saturations with exercise (Hamutcu et al. 2004).

Of all the indications for the use of ECMO those patients with acute respiratory failure secondary to meconium aspiration syndrome appear to have the best outcome both in terms of survival rate and subsequent respiratory health (Bahrami and van Meurs 2005). Venovenous ECMO has been shown to be an optimum therapeutic modality for meconium aspiration syndrome (Kugelman et al. 2005).

Cerebrovascular complications result from microemboli, with microinfarcts and the increased risk of hemorrhage. Studies have demonstrated dramatic effects on cerebral perfusion during VA ECMO, with marked reduction in arterial flow particularly if there is any obstruction in the venous cannula (Weber and Kountzman 1996). Neurodevelopmental defects may be manifest in survivors and can result from the primary pathology and from the complications of ECMO therapy (Graziani et al. 1997). The reported frequency of brain abnormality as identified by various neuroimaging modalities varies between 28% and 52% of ECMO-treated neonates, and is associated with functional deficiency in childhood (Bulas and Glass 2005). However, it appears that most newborn infants who received ECMO therapy for acute respiratory failure (of which the majority will be meconium aspiration syndrome) have normal neural developmental screening assessment at 1 to 1½ years of age (Khambekar et al. 2006).

Cardiovascular complications, including myocardial stun and infarction, have an adverse effect on survival during ECMO (Becker et al. 1998). Hemorrhage is a significant complication in up to a third of patients, and sepsis is predictably a concern.

Mechanical problems related to the ECMO circuit have been reported in up to 20% of cases (Extracorporeal Life Support Organization Registry 1990).

Extracorporeal membrane oxygenation is a labor-intensive and expensive therapeutic modality that should be limited to a few dedicated centers.

Nitric Oxide

The addition of nitric oxide to ventilating gases to promote vascular relaxation in the pulmonary vascular bed is increasingly being employed in neonates with persisting pulmonary hypertension. Nitric oxide was identified as the endothelium-derived vasodilator factor by Ignarro and coworkers (1987). Subsequently its central role in control of vascular tone and the related chemistry have been defined. It is a major factor in the transition from the high-resistance state of the fetal pulmonary circulation to the low-resistance adult state (Gaston et al. 1994; Miller 1995).

The efficacy of inhaled nitric oxide in reducing pulmonary vascular resistance is unquestioned, but the effects are frequently short-lived and pulmonary hypertension recurs after cessation of nitric oxide therapy. A recently reported multicenter randomized control trial of inhaled nitric oxide therapy for premature neonates with severe respiratory failure has concluded that the treatment does not decrease the rate of death or rate of development of bronchopulmonary dysplasia in critically ill premature infants weighing less than 1400 g (van Meurs et al. 2005).

There are several observed and theoretical concerns regarding the toxicity of nitric oxide. It binds avidly to hemoglobin, where it is quickly inactivated with the resultant formation of methemoglobin, inorganic nitrate, and nitrite (Kinsella and Abman 1993). Under certain conditions nitrogen dioxide and peroxynitrite free radicals can form (Beckman et al. 1990). Nitrogen dioxide is toxic to lung tissue and can cause the development of pulmonary edema. Peroxynitrite, by its oxidant capacity, damages lipid membranes and surfactant (Haddad et al. 1993; Kooy et al. 1995; Matalon et al. 1996) and binds to nucleic acid and proteins at tyrosine residues, forming nitrotyrosine with a theoretical risk of teratogenicity and

mutagenicity (van der Vliet et al. 1994; Tamir et al. 1996). The real risk of long-term sequelae, particularly of a teratogenic and mutagenic/carcinogenic nature, is likely to be small but is as yet undefined. The passage of time will be the test in this regard.

Liquid Ventilation

Perfluorocarbons dissolve large quantities of oxygen and carbon dioxide at atmospheric pressure. At normal atmospheric pressure conditions a saturated solution of perfluorocarbon contains approximately 15 volume percent of oxygen (Sargent and Seffi 1970). Ventilation by instillation of perfluorocarbon into the airways is being increasingly employed in neonatal and adult intensive care where there is a need for respiratory support and augmentation. The treatment appears to be remarkably devoid of complications, and the histological appearances of liquid ventilated lung tissue are remarkable for their "normality" as a result presumably of the removal of exudate and damaging cytokines, the expansion of alveolar saccules with enhancement of the blood–gas interface surface area, and the avoidance of the barotrauma associated with positive pressure ventilation. Hemodynamic embarrassment and lactic acidosis have been reported during liquid ventilation (Lowe et al. 1979; Sivieri et al. 1981). However, there is now a very large literature reporting the use of liquid ventilation in a number of clinical scenarios both in children and adults, and there is no evidence of any significant deleterious consequence related to the treatment alone.

Infection

The subject of infection is dealt with in detail elsewhere in this book (see Chapter 16). Unlike in the fetus, which is protected in utero to a substantial degree, the neonatal period represents the time of greatest vulnerability to infection. Passage through the birth canal exposes the neonate to a complex bacteriological and virological environment with numerous virulent pathogens, some of which colonize the maternal genital tract, for example, ß-hemolytic streptococcus.

The premature neonate or a baby born with hypoxemia is at particular risk. A combination of an immature immunological system and other major system functional deficits increases the risk of infection. The wide range of therapeutic measures employed in the neonatal intensive care environment, for example, endotracheal intubation and the insertion of vascular cannulae, breach the fragile local defense mechanisms of the neonate and create portals of entry for infectious agents, which almost invariably are more likely to be pathogenic than those microorganisms that would be encountered outside the hospital environment.

Complications of Pharmacological Interventions in Neonatal Lung Disease

Surfactant Therapy

The administration of exogenous surfactant given either prophylactically or as a "rescue" therapy has had a considerable impact on the incidence and severity of respiratory distress syndrome and chronic lung disease in premature neonates. The results of rescue therapy are less dramatic than those of prophylactic therapy. Toxicity from the various forms of animal and artificial surfactant appears to be minimal, and in particular antibodies are not formed to the bovine and porcine animal-derived surfactants (Chida et al. 1991).

Surfactant therapy is also effective in the management of other forms of neonatal respiratory disease in which the efficiency of endogenous surfactant is altered by aspirated material or inflammatory exudate. The cholesterol, free fatty acids, and bilirubin in meconium show a dose-dependent interference with surfactant function that can be overcome by endogenous surfactant therapy. Animal studies have shown similar effects of endogenous surfactant in bacterial-mediated inflammation of the lungs (Finer 2004).

The sole significant complication is a higher incidence of massive pulmonary hemorrhage particularly following the use of Exosurf in small babies weighing less than 800 g (Stevenson et al. 1992; Van Houten et al. 1992). A meta-analysis of

29 trials was conducted by Raju and Lanenberg (1993) and confirmed an association between massive pulmonary hemorrhage and synthetic but not natural surfactant. The large OSIRIS study showed pulmonary hemorrhage to occur in 5% to 6% of babies treated with Exosurf (open study of infants at high risk of or with respiratory insufficiency (OSIRIS) Collaborative Group 1992).

Indomethacin

Recovery from otherwise uncomplicated respiratory distress syndrome is complicated by significant shunting through a patent ductus arteriosus in approximately 20% of cases (Ellison et al. 1983). Indomethacin is routinely utilized to close the ductus arteriosus and is successful in 75% to 80% of cases (Gersony et al. 1983). The drug has several side effects including reduction of renal output and fluid retention (Cifuentes et al. 1979). In addition it has been shown to be associated with an increased risk of gastrointestinal hemorrhage (Friedman and Fitzpatrick 1980) and disorders of coagulation (Corazza et al. 1984).

A potentially more serious consequence of indomethacin therapy is mediated by its effect on cerebral hemodynamics. The drug causes a marked decline in cerebral blood flow, cerebral oxygen delivery, and cerebral blood volume, and may also reduce the oxygenation of the brain (Pryds et al. 1988; Edwards et al. 1990). The development of cystic brain lesions and interventricular hemorrhage have also been associated with indomethacin therapy (Norton et al. 1993; Souter et al. 1998). A randomized controlled trial confirmed the effects of indomethacin on cerebral blood flow and demonstrated that ibuprofen, while having a similar therapeutic benefit in closure of a patent ductus arteriosus, was not associated with disordered cerebral hemodynamics (Patel et al. 2000).

Antioxidant Therapy

Vitamin E and superoxide dismutase treatment have been used in the management of evolving chronic lung disease. Trials have shown no benefit of vitamin E in the prevention of bronchopulmonary dysplasia, but the incidence of neonatal sepsis and necrotizing enterocolitis (NEC) has been shown to be higher in neonates receiving vitamin E therapy for 8 days or longer (Johnson et al. 1985). Vitamin E decreases the oxygen-dependent intracellular killing ability of neutrophils, and may result in a decreased resistance of preterm infants to infective organisms (Johnson et al. 1985).

A similar theoretical risk arises with the use of antioxidant superoxide dismutase treatment, which may affect the bactericidal activity of neutrophil polymorphs.

Complications of Chest Drains

Perforation of the lung by chest drains is not uncommon and has been reported in approximately 25% of cases in some studies (Moessinger et al. 1978). This complication is more likely to occur in situations of poor pulmonary compliance and with lungs that become full and voluminous as a result of significant interstitial air leak and intraalveolar hemorrhage. The avoidance of sharp trocar insertion and utilization of blunt dissection for the insertion of chest drains minimizes the incidence of direct pulmonary perforation by the drain tube. Injury to the thoracic duct causing chylothorax (Kumar and Belik 1984), cardiac trauma with tamponade (Quak et al. 1993), and phrenic nerve injury (Ayalon et al. 1979; Marinelli et al. 1981; Arya et al. 1991) are also reported.

Direct lung puncture can give rise to bronchopleural fistula formation, which may require surgical repair (Berger and Gilhooly 1993).

Complications Related to Monitoring, Vascular Cannulation, and Blood Sampling

Intermittent and continuous monitoring of multiple parameters are an essential feature in neonatal intensive care. The dedicated and specially trained neonatal intensive care nurse is a key figure in the process and is only assisted by the various monitoring devices utilized to display and record cardiorespiratory function and other modalities. Inherent with any system involving machines is the possibility that as a result of some deficiency in setting up the equipment or some equipment failure, inappropriate information can be proffered to nursing staff and medical staff. It is important that all monitoring devices are

checked regularly and that all staff members are aware of the common system faults that may arise.

Arteries

Arterial blood sampling and monitoring of blood gases are an essential part of neonatal intensive care. The target range for paO_2, $paCO_2$, and pH requires relatively tight control if the deleterious consequences of hypoxemia, hyperoxemia, alkalosis, and acidosis are to be avoided.

The development of indwelling arterial lines permits neonatologists to take frequent samples or to continuously monitor a number of parameters. The routine method of obtaining arterial blood is to insert an umbilical arterial catheter (UAC). This is usually straightforward in the early days after delivery, but as with all vascular cannulation there is the potential for endothelial trauma and associated thrombosis. Resultant thrombosis in the aorta or iliac arteries is common and is reported with a frequency of between 24% and 95% in infants investigated by angiography and seen in 3.5% to 48% of cases coming to necropsy (Westrom et al. 1979). In my experience a small amount of adherent thrombus can be identified in relation to almost every umbilical arterial catheter, but I have seen only one serious thrombosis with ischemic damage to related organs in the past 10 years. Usually the thrombus is small and associated with the external wall of the catheter often adherent to the catheter tip. Thrombosis is more commonly seen in catheters with a side hole, and this is thought to be related to the presence of a dead space between the side hole and end hole of the catheter tip.

Given the frequency with which umbilical arterial cannulae are inserted in neonates, it is comforting to note that the incidence of serious complication is very low if attention is given to the optimum positioning of the catheter in the aorta and if recognized standard procedures of catheter management are followed. The danger area for the risk of serious embolization of intraabdominal organs is in the zone from T12 to L3/4 (Phelps et al. 1972). In this area the arteries to the kidneys and intestines take origin. The theoretical risk of embolization from catheters that are positioned above T12 with subsequent increased risk of NEC do not appear to present as a clinical problem (Kempley and Gamsu 1992). The low positioning of the cannula tip can give rise to obstruction of blood flow to the lower limbs (Mokrohisky et al. 1978). Umbilical arterial catheters are associated with modest elevation of blood pressure, which is not thought to give rise to any long-term sequelae (Emery and Greenough 1992a,b).

Significant complications of umbilical arterial cannulation, although rare, are very serious and include aortic thrombosis (Vailas et al. 1986) (Fig. 17.11); thrombotic episodes affecting the lower limbs (Goetzman et al. 1975) (Fig. 17.12), kidneys (Marsh et al. 1975), and the gastrointestinal tract (Gupta et al. 1968) (Fig. 17.13); damage to the urachus with urinary leakage (Dmochowski et al. 1986); development of aortic aneurysmal dilatation (Drucker et al. 1986); and nerve damage, including the development of paraplegia (Haldeman et al. 1983; Fok et al. 1986).

Gluteal skin necrosis as a complication of umbilical arterial catheterization was described

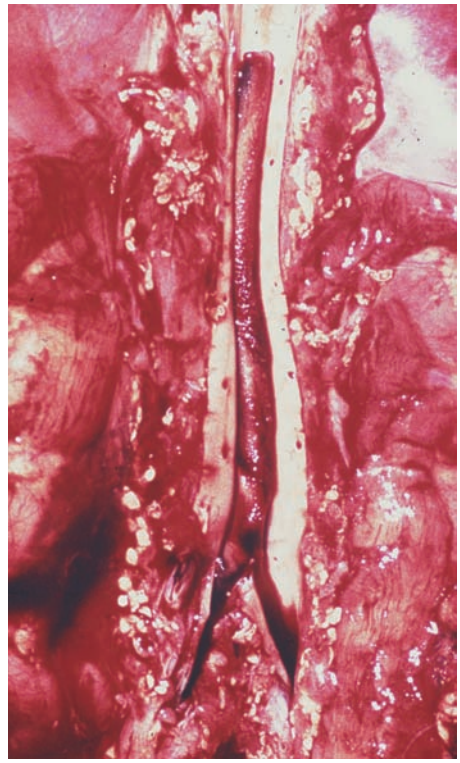

Figure 17.11. Massive aortic thrombosis following umbilical arterial catheterization.

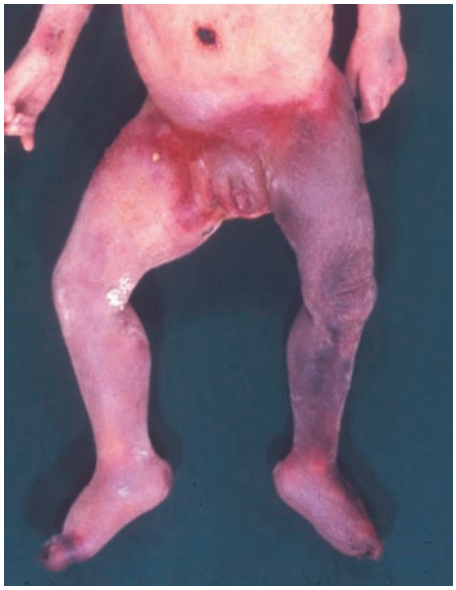

Figure 17.12. Gangrene of the perineum and lower limb caused by massive aortic thrombosis.

by Mann (1980), but others have implicated the prolonged contact with alcohol-based skin cleansing agents as a causative factor (Wilkinson et al. 1981; Harpin and Rutter 1982).

Cannulation of peripheral arteries is occasionally utilized when an umbilical arterial cannula cannot be inserted. Peripheral arterial cannulae unlike those inserted via the umbilical artery should not be used for infusion purposes, as this gives rise to arterial spasm. It is vital to check that there are good collateral blood supplies distally before cannulation of radial, ulnar, or posterior tibial arteries. Simmons et al. (1978) reported ischemic brain injury secondary to cannulation of the temporal arteries presumably as a result of arterial spasm in the territory of the ipsilateral external carotid artery. Lin et al. (2001) report their experience of complications resulting from femoral arterial catheterization in pediatric patients. Nonischemic complications had a good outcome, but a small proportion of children presenting with ischemic complications did not regain normal circulation to the limb despite surgical interventions, although no limbs were lost.

Gamba et al. (1997) reported a neonatal unit experience of vascular injuries in a study group of 2898 neonates. The incidence of significant pathology, for example, arteriovenous fistulas, carotid artery trauma, and limb ischemia, was strongly correlated with birth weight; 2.6% of low birth weight babies suffered significant iatrogenic vascular pathology as compared with 0.3% of neonates weighing more than 1500 g.

Intermittent arterial puncture should be less frequently required in neonatal intensive care where continuous monitoring catheters or umbilical arterial catheters permitting intermittent sampling are in situ. The risk of introduction of infection into the repeated arterial puncture area and also of direct vascular injury is obvious. Fortunately, these complications are relatively rare as is the risk of distal ischemic secondary to arterial spasm.

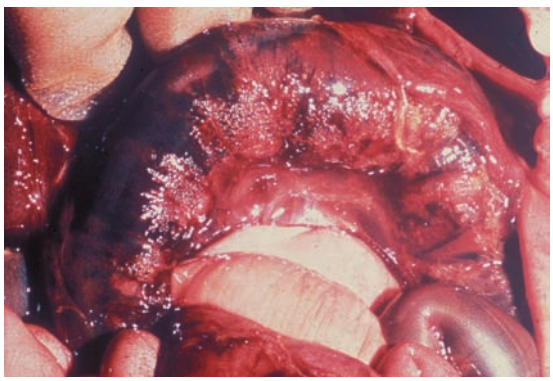

Figure 17.13. Infarction of the colon following aortic thrombosis.

Veins

Cannulation of umbilical veins is relatively rarely performed because of the high frequency of complications (Figs. 17.14 and 17.15). Umbilical vein thrombosis was extremely common following catheterization and particularly frequent after the infusion of hypertonic solutions (Kitterman et al. 1970). The frequency and pattern of thrombotic and embolic complications were related to the positioning of the end of the catheter. Typically the umbilical venous cannulas are positioned in the right atrium but may occasionally be in the thoracic inferior vena cava. Malposition of the cannula tip in the portal vein with subsequent

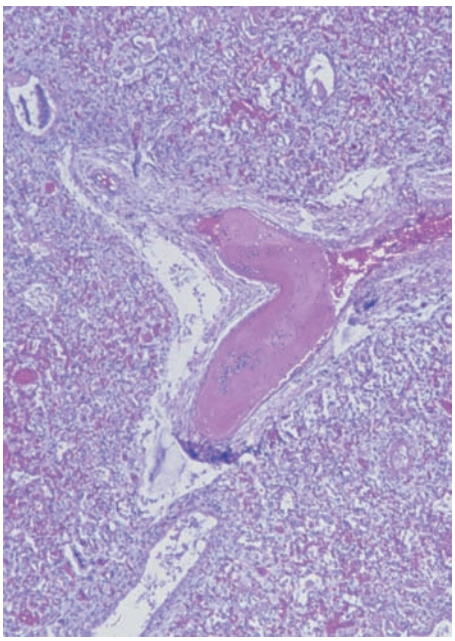

Figure 17.14. Thromboembolus straddles a pulmonary arterial bifurcation following umbilical venous cannulation.

portal vein thrombosis and subsequent hepatic necrosis was reported by Larroche (1970). More chronic consequences of portal vein thrombosis included portal hypertension with splenomegaly

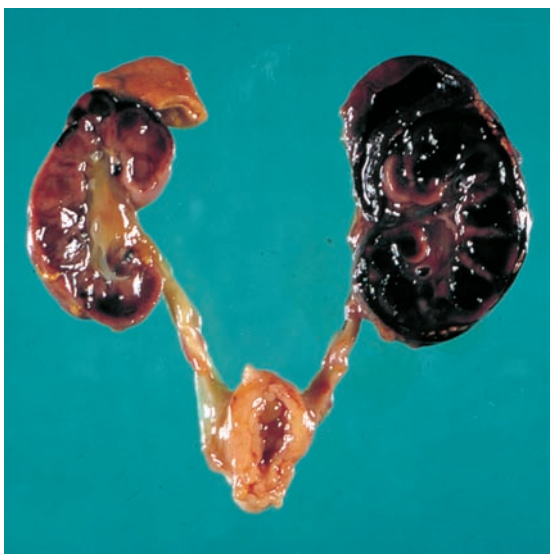

Figure 17.15. Venous infarction of the left kidney secondary to inferior vena caval thrombosis after umbilical venous catheterization.

or hematemesis (Junker et al. 1976; Lauridsen et al. 1978).

More commonly venous catheters are inserted in systemic veins and positioned in the subclavian and superior vena cava territories for the purposes of parental alimentation. The principal complication with these lines appears to be a high risk of bacterial and fungal colonization with dissemination of infection. Thrombosis related to the tip of the cannulae and propagation of the thrombus into the superior vena cava and heart are also not uncommon. An infrequent but well-recognized complication of venous catheterization is perforation of the myocardium.

Venepuncture is associated with a number of potential hazards. Inadvertent puncture of the brachial artery with resultant false aneurysm formation is described by Rey et al. (1987). The potential for introduction of infection in venepuncture sites is obvious.

Other Causes of Complications

Burns

Neonatal skin is more sensitive to burning than is adult skin. Burns have been reported in instances of prolonged exposure to warming devices at temperatures as low as 42°C (Mohrenschlager et al. 2003), and second-degree burns have been reported following resuscitation under infrared heating lamps (Simonsen et al. 1995).

Topical Preparations

The high surface area to volume ratio of small neonates combined with the relative fragility and poor keratinization of neonatal skin increase the potential for absorption of topical preparations.

Hexachlorophene

A classical example of this risk was hexachlorophene, which was formerly used as a bacteriostatic agent and was applied as a whole-body application for cleansing purposes. Abnormalities of the central nervous system were first identified in experimental animals in the form of spongiform degeneration after repeated applications of

hexachlorophene. Similar changes were identified by Powell et al. (1973) in the brains of six preterm infants who had received at least four whole body exposures to hexachlorophene. Shuman et al. (1974) found similar abnormalities in the brains of 17 of 240 babies who were all of low birth weight and who had experienced repeated applications of 3% hexachlorophene solution. This experience should serve as a warning of the special conditions of neonatal skin.

Alcohol-Based Cleansing Solutions

Wilkinson et al. (1981) and Harpin and Rutter (1982) identified the consequences of prolonged exposure of the skin to alcoholic solutions of chlorhexidine and industrial methylated spirits. These exposures resulted in superficial skin necrosis in the areas exposed to the alcoholic solutions (Fig. 17.16). Harpin and Rutter (1982) also demonstrated the absorptive capacity of the skin by finding high blood levels of ethanol and methanol in some of the babies exposed to methylated spirits.

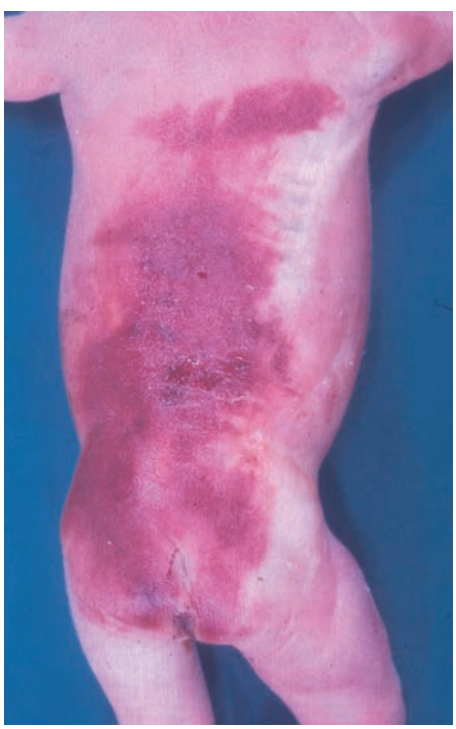

Figure 17.16. Dorsal cutaneous necrosis following prolonged contact with an alcohol-based skin-cleansing agent.

Systemic Treatments

The major risk with regard to drugs administered systemically is inadvertent computation errors and subsequent drug overdose (Koren et al. 1986). This is undoubtedly a much more frequent occurrence in pediatric centers than the literature would indicate (Jonville et al. 1991).

Antibiotics

Antibiotics are a major cause of drug-induced renal disease as a result of direct toxicity or immunologically mediated injury. Antibiotics are widely used in neonatal intensive care, for example, aminoglycosides, glycopeptide, β-lactams, etc., and all show varying potential for nephrotoxicity. In most instances this is reversible on discontinuation of treatment (Fanos and Cataldi 1999).

Diuretics

Diuretics such as furosemide, chlorothiazide, and spironolactone are frequently used in the management of chronic lung disease. Furosemide can provide dramatic improvements in lung compliance and reduction of airways resistance (Kao et al. 1983; Najak et al. 1983), and prolonged therapy with chlorothiazide and spironolactone has been reported to improve the outcome in patients with severe bronchopulmonary dysplasia (Albersheim et al. 1989).

Furosemide administration may cause hyponatremia and hypocalcemia. Chronic diuretic therapy is associated with hypercalcuria, renal calcification, and nephrolithiasis. The renal calcification is composed of calcium oxalate and calcium phosphate (Hufnagle et al. 1982). This may be associated with demineralization of bones. Renal calcification is more common in immature infants receiving longer courses of treatment and has been reported as occurring in up to 48% of infants receiving long-term furosemide therapy (Robinson and Cox 1986). The calcification usually resolves spontaneously following discontinuity of treatment, but active therapy with chlorothiazide may be utilized to increase urinary calcium excretion and promote the resolution of calcification (Hufnagle et al. 1982).

Other complications of chronic diuretic therapy include hyperchloremia (McCann et al. 1985),

metabolic alkalosis (De Rubertis et al. 1970), and ototoxicity (Rybak 1982; Salamy et al. 1989).

Steroids

Steroids are utilized in the treatment of chronic lung disease and give rise to improvements in lung function, although effects on survival and the long-term outcome are less clear (Cummings et al. 1989; Collaborative Dexamethasone Trial Group 1991).

Numerous side effects of steroid therapy have been reported, and it appears important that sepsis and patency of the ductus arteriosus be excluded prior to initiation of treatment. Depression of immune function is a potentially serious consequence of steroid therapy, but studies have provided conflicting results as to the significance in neonates (Gunn et al. 1984; Ng et al. 1990). Steroids are associated with gastrointestinal complications including hemorrhage, peptic ulceration, and gastric perforation (Ng et al. 1991; O'Neil et al. 1992). Significant hypertension can follow steroid therapy and persist for several days after treatment has been discontinued (Emery and Greenough 1992; Greenough et al. 1992). Hypertensive encephalopathy has been associated with steroid-induced hypertension. Dexamethasone has been associated with a transient myocardial hypertrophy (Werner et al. 1992) and hypertrophic obstructive cardiomyopathy (Brand et al. 1993). The cardiac pathology resolved completely after cessation of treatment. Dexamethasone is also known to have a catabolic effect in preterm infants, causing a rise in urea secondary to catabolism of muscle tissue (Brownlee et al. 1992; Williams and Jones 1992). The risk of adrenal suppression following prolonged use of exogenous steroid therapy in premature babies appears to be very small (Rennie et al. 1989). However, suppression of the hypothalamic pituitary access at the pituitary level has been identified in prolonged dexamethasone therapy (Rizvi et al. 1992).

Tolazoline

An α-adrenergic blocking agent used in the management of pulmonary hypertension, tolazoline is associated with the development of gastrointestinal ulceration and hemorrhage (Ward 1984; Abu-Osba 1991) (Fig. 17.17).

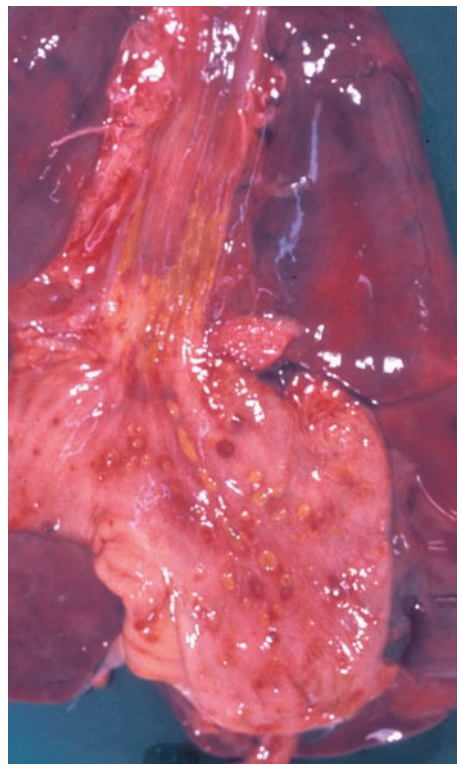

Figure 17.17. Multiple discrete ulcers in the gastric mucosa. At necropsy, the stomach and duodenum were filled with blood.

Prostaglandin E_1

This drug is used to maintain the patency of ductus arteriosus in neonates with cyanotic congenital heart disease. Heffelfinger et al. (1987) reported the development of pulmonary arteritis following prostaglandin E_1 therapy and proposed a causal relationship. Oeda et al. (1980) reported the development of cortical hyperostosis following long-term administration of prostaglandin E_1 in infants with cyanotic congenital heart disease.

Total Parenteral Nutrition

Intravenous alimentation is widely used in pediatric practice, most particularly in neonates with gastrointestinal pathology including NEC. Increasingly it is being employed in neonatal intensive care units to supplement the oral feeding of very small neonates. Most neonates with severe respiratory illnesses have ileus and delayed gastric emptying. This plus the high

frequency of gastroesophageal reflux makes enteral nutrition potentially problematic.

Intravenous alimentation in the form of either supplementation of enteral feeding or as total parenteral nutrition (TPN) involves the intravenous infusion of solutions of amino acids, sugar, and lipid emulsion with additional vitamins and trace elements added. Amino acid and calcium infusions are intensely irritant if they leak outside the vascular compartment.

The most frequent complication relates to infection by bacteria and fungi colonizing the intravenous line.

Disturbances of liver function and cholestasis are well-recognized complications of prolonged TPN. Peden et al. (1971) was the first to draw attention to the hepatic complications of TPN in infants. The development of TPN-associated cholestasis is related to the duration of treatment and correlates inversely with the gestational age and birth weight. It is a diagnosis of exclusion, given the numerous other causes of neonatal cholestasis that are possible. The morphological appearances are not specific and are variable (Balistreri and Bove 1990; Benjamin 1981; Cohen and Olsen 1981). Typically there is marked cholestasis affecting liver cells and canaliculi and cholestatic hepatocyte rosettes are frequently present (see Fig. 19.25 in Chapter 19). Bile plugs may be present in interlobar bile ducts. Steatosis is infrequent. The portal tracts usually exhibit a very light mixed inflammatory infiltrate. Prolonged therapy is associated with a periportal ductular reaction and progressive fibrosis.

The use of fat emulsion in intravenous alimentation is associated with additional specific and potentially very serious adverse consequences. Barson et al. (1978) first described pulmonary lipid embolism in patients who received lipid infusions. Subsequently lipid infusions were shown to be associated with a fall in paO_2 (Pereira et al. 1980). Randomized prospective control trials in preterm neonates receiving intralipid demonstrated longer requirement for oxygen therapy and intermittent positive pressure ventilation and also a higher rate of development of chronic lung disease (Hammerman and Aramburo 1988; Sosenko et al. 1993). Cooke (1991) showed that intravenous lipid was a precursor of chronic lung disease in low birth weight infants.

The reticuloendothelial system takes up lipid droplets in macrophages following lipid emulsion administration (Fig. 17.18). In animals this has

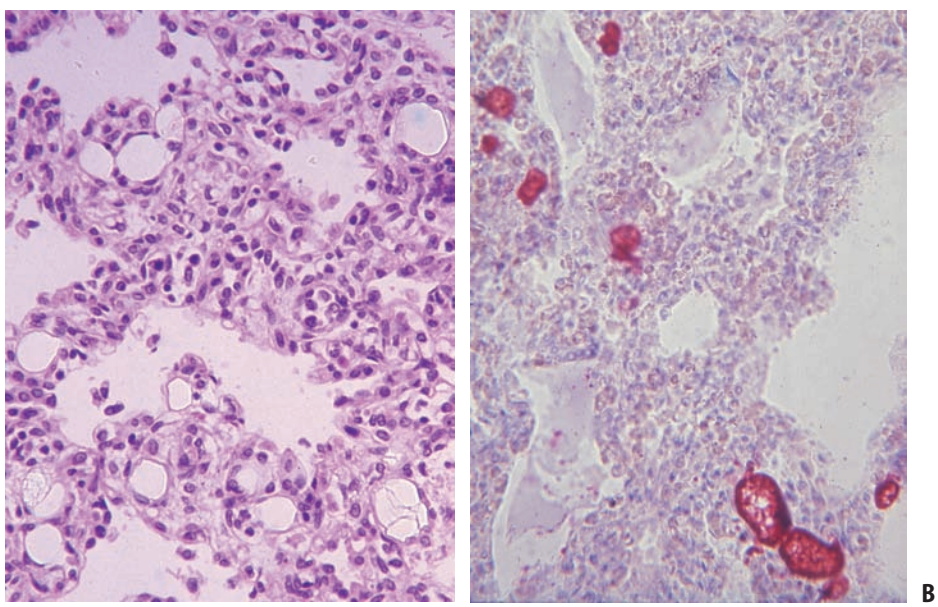

Figure 17.18. Rounded lipid droplets fill pulmonary capillaries following lipid emulsion infusion. (A) Hematoxylin and eosin. (B) Oil red O. (Courtesy of Dr. A.J. Barson, Manchester, England.)

been shown to result in defective neutrophil and macrophage function (Fischer et al. 1980), and Freeman et al. (1990) showed that intralipid treatment may increase the risk of staphylococcal epidermidis sepsis. Yu (1992) has reviewed the subject of parenteral nutrition in the newborn.

Blood Transfusion

Potential complications related to blood transfusion are numerous. They range from technical and procedural errors in cross-matching, insertion intravascular lines, and problems with volume, and the potential for disturbance of body temperature, to more specific factors within the transfusion itself (Macpherson et al. 1988).

Infection

Today, no blood product can be regarded as entirely free from the risk of infection with any of a number of viral agents [cytomegalovirus (CMV), hepatitis viruses, HIV], and in recent times the question of the potential risk of exposure to prions, the infectious agent in spongiform encephalopathy [Creutzfeldt-Jakob disease (CJD) and new-variant CJD], has been raised.

However, for all practical purposes the risk of infection is extremely low for patients in the developed world as a consequence of rigorous screening of blood donors and donations for infectious agents (Chamberland et al. 1998; Gresens and Holland 1998; Pratti et al. 1998). Recipients of infected blood have a high risk of established infection. Dike et al. (1998) reported that 76% of a cohort of patients who received hepatitis C virus (HCV)-positive blood prior to the establishment of the 1991 screening system became infected as evidenced by the detection of HCV RNA in the recipients of the blood donations.

The position in developing countries is less assured, with high rates of infectivity in the population in general and in donations (Aggarwal et al. 1997). The cost of screening tests is a serious burden in many countries, and the risk of infection from blood products is significant.

Transfusion-transmitted CMV infection is potentially serious in immunocompromised patients. Neonates, particularly those who are premature, have suboptimal immune systems, and with the additional stresses of other neonatal disorders are at risk of serious illness rather than the more usual asymptomatic seroconversion. Again screening of blood given to immunocompromised patients significantly reduces the risks (Bowden 1995).

Graft-Versus-Host Disease

Transfusion-associated graft-versus-host disease (GVHD) is rare but carries a very high mortality. The condition results from the proliferation of donor T lymphocytes in an immunocompromised host incapable of their elimination. Irradiation of blood products is the method currently employed to inhibit the proliferative capacity of T lymphocytes in blood, and this is routine if a patient is known to be immunocompromised. However, I have seen two cases where the diagnosis of immunodeficiency followed the development of GVHD after blood transfusion. Both cases were fatal. The clinical presentation is typical of GVHD in other clinical settings, for example, bone marrow transplantation (Brubaker 1986; Anderson and Weinstein 1990; Gresik 1996).

Skeletal Abnormalities

Rachitic changes in the ribs of premature infants with respiratory pathology characterized by expansion of the epiphyses and costochondral junctions, angulation of the ribs, and occasional rib fractures are well described (Chudley et al. 1980; Oppenheimer and Snodgrass 1980) (Fig. 17.19). These abnormalities have been attributed to the results of low intake of calcium and vitamin D, with these deficiencies being accentuated by the administration of sodium bicarbonate and the use of furosemide, which increases calcium excretion. Venkataraman et al. (1987) demonstrated that a number of hormones and other agents likely to cause hypocalcemia are present in very sick low birth weight neonates. It is likely, therefore, that the etiology of calcium depletion from the ribs is multifactorial, although calcium supplementation and dietary supplementation will alleviate and ameliorate the pathology. These skeletal abnormalities should be rare in modern neonatal intensive care practice.

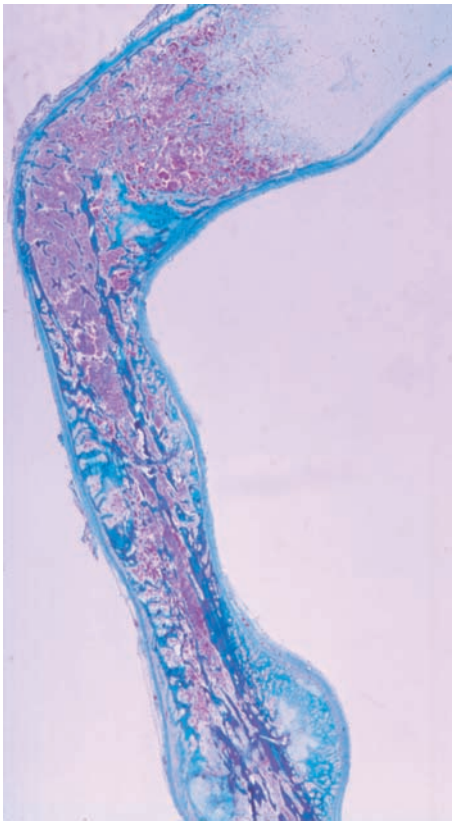

Figure 17.19. Rib with irregularity and expansion of the osteochondral junction, angulation, and healing fractures are features of rickets induced by dietary deficiencies.

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18
The Alimentary Tract and Exocrine Pancreas

Jean W. Keeling

Gastrointestinal abnormality is common in both the fetus and the neonate. Gastrointestinal malformations are diagnosed in fetal life with increasing frequency, enabling delivery in units with appropriate facilities. Gastrointestinal malformations, both isolated and syndromatic, are encountered at necropsy, although only a small proportion are, in themselves, fatal. Malformations and postnatally acquired disease contribute to neonatal morbidity and mortality, comprising most of the specimens received from neonatal surgical units. An understanding of gastrointestinal development, the range of pathological abnormalities, their associations, and their significance makes an important contribution to clinical management of the affected individual and family.

Development

The gut derives from that part of the secondary yolk sac that becomes enclosed by the head, tail, and lateral folds of the embryonic plate at around the 20th day of development. The head and tail folds enclose the fore- and hindgut, respectively. The intermediate portion of the intraembryonic yolk sac forms the midgut, which connects through a wide communication with the extraembryonic part of the secondary yolk sac (the definitive yolk sac). This communication becomes progressively reduced by better definition of the head, tail, and lateral folds of the embryo (Fig. 18.1); by 28 days the communication is via a narrow vitelline duct, and the umbilical cord is clearly visible. At about this time the primitive foregut and hindgut come into contact with rostral and caudal invaginations, the stomodeum and proctodeum. The omphalomesenteric duct usually becomes obliterated by the 10th week of embryonic life.

Most of the epithelium and glands of the digestive tract originate from embryonic endoderm, while the epithelium of the cranial and caudal ends is derived from the stomodeum and proctodeum, respectively. Early in the 3rd week the endoderm grows dorsally and becomes pinched off to form the notochord, which induces the formation of the vertebral column. In the foregut, two lateral ridges appear and grow toward each other in the midline, separating an anterior diverticulum, which becomes the larynx and trachea, from the esophagus. The stomach appears as a fusiform expansion toward the caudal end of the foregut in the cervical region of the embryo. The esophagus elongates rapidly during the 6th to 7th weeks, reaching its final relative length by the end of this time. The original lining of the esophagus is simple ciliated pseudostratified columnar epithelium, which is replaced progressively by squamous epithelium from the 5th intrauterine month. Swallowing has been observed in utero as early as 16 to 17 weeks' gestation and is probably important in the maintenance of amniotic fluid volume in later pregnancy.

At about the 4th week the intestine is almost a straight tube suspended by a mesentery from the dorsal body wall. Because the abdominal cavity is too small to accommodate the enlarging liver and growing intestine the midgut herniates into the extraembryonic celom in the base of the umbilical

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This chapter is based in part on Chapter 15 "The Alimentary Tract and Exocrine Pancreas" written by Dick Variend in Fetal and Neonal Pathology 3rd Edition. Copyright Springer-Verlag, London 2001.

cord during the 6th week. Between 9th and 10th weeks, the midgut withdraws from the umbilical cord and reenters to the abdominal cavity. A 180-degree counterclockwise rotation occurs around the axis of the superior mesenteric artery late in this stage, and, as a result of a further 90-degree counterclockwise rotation, the cecum lies in the right upper quadrant. From the 12th week to birth, the cecum descends to its final location in the right lower quadrant.

Rudimentary villi appear at 8 weeks' gestation and early crypts are present at 12 weeks. At the 5th week of gestation a group of neuroblasts appears bilaterally along the vagal trunks at the level of the pharynx (Okamoto and Ueda 1967). They appear around the esophagus at 6 weeks and reach the cephalic limb of the midgut by 7 weeks. By 12 weeks they are present throughout the alimentary tract down to the rectum. The early neurons appear first in the myenteric plexus, and then migrate across the circular muscle to populate the submucosa. A more caudal neural crest origin for the distal enteric neurons has been suggested (Andrew 1971). By 22 weeks' gestation the small intestine is histologically mature.

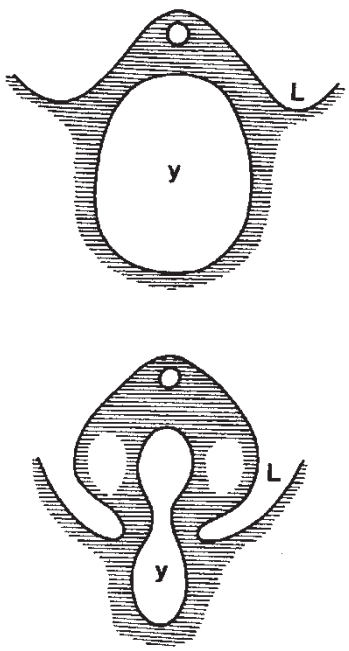


Figure 18.1. Transverse slice through embryo illustrates diagrammatically the vitelline duct. y, yolk sac; L, lateral fold.

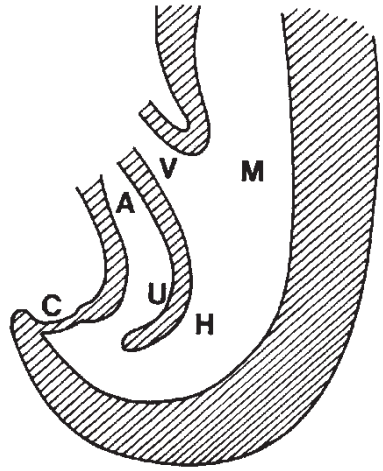


Figure 18.2. Division of the cloaca into urogenital sinus and rectum. U, urorectal septum; A, allantois; H, hindgut; C, cloacal membrane; V, vitelline duct; M, midgut.

The cloaca, the dilated terminal part of the primitive hindgut, receives the allantois ventrally and the mesonephric ducts laterally. The urorectal septum develops in the angle between the allantois and the midgut and divides the cloaca into urogenital sinus and rectum (Fig. 18.2). This division is normally complete by the end of the 6th week of gestation. The membrane covering the anal canal disappears by week 9, establishing communication between the digestive tract and amniotic cavity.

Oral Cavity

Cleft Lip and Palate

Clefts of the lip or palate (CL/P) are among the commonest birth defects worldwide, affecting approximately 1 in 1000 live births (Schutte and Murray 1999). Birth prevalence varies between populations by up to fourfold (1 in 500 to 1 in 2000) (Scott et al. 2005). The majority of these defects are nonsyndromic, although gene studies indicate a significant overlap between syndromic and nonsyndromic cases (Stanier and Moore 2004). The genetics of CL/P is complex (Colbourne 2004), but environmental factors, such as sodium valproate, maternal folate deficiency, and familial diabetes, are important. One of the challenges is to unravel the interrelationships between

teratogens and individual genes (Carinci et al. 2005). High parental age is also a risk factor (Bille et al. 2005).

Clefts of the lip or palate can be unilateral or bilateral (Fig. 18.3). The pathologist is likely to encounter a high proportion of syndromic cases. It is frequent in trisomy 13 and 4p deletion syndromes and less common in trisomies 13 and 18 (see Fig. 2.14 in Chapter 2) and triploidy (Jones 1997).

Disruptive oral clefts are often part of the amnion disruption sequence. Midline clefting can accompany holoprosencephaly and short-rib/polydactyly syndromes such as Majewski and Beemer-Langer (see Chapter 27).

Microstomia comprises severe hypoplasia of the mandible accompanied by hypoplasia of the mouth. It is frequently seen in circumstances where fetal movement is restricted, such as multiple pterygium sequence, Pena Shokier phenotype, and Moebius syndrome (De Serpa Pinto et al. 2002), serving as a reminder to sample adequately the central nervous and musculoskeletal systems for histology and immunohistochemistry. It may accompany restrictive dermopathy (Armbrust et al. 2005), trisomy 18, and fetal valproate syndrome, as well as craniofacial malformations (McKenzie et al. 2002).

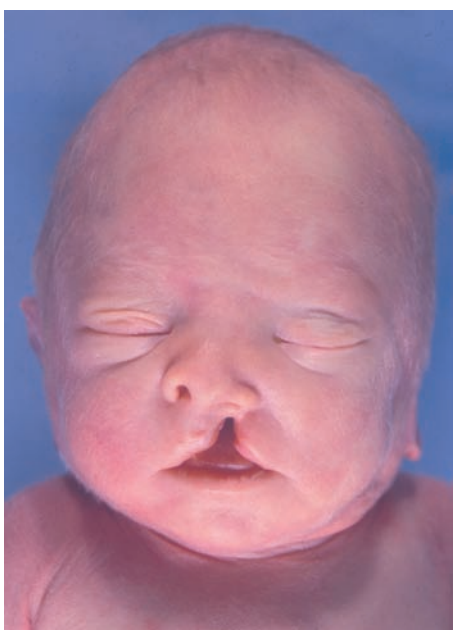


Figure 18.3. Unilateral cleft lip/palate as an isolated anomaly (see also Figure 2.14 in Chapter 2.)

A broad oral cleft is known as *macrostomia*. Both of these anomalies are frequent components of malformation syndromes (Jones 1997), particularly when there are facial dysmorphisms (Stevens and Sargent 2002).

The Tongue

The tongue may be abnormally large (macroglossia); causes include Beckwith–Wiedemann syndrome, trisomy 21, glycogenosis type 2, and lymphangioma.

Cysts and Tumors

A *lingual thyroid* is a localized midline mass in the posterior third of the tongue that results from maldescent of the thyroid primordium. Thyroglossal duct remnants, commonly manifesting as cysts, may also occur at this site. Dermoid cysts rarely present in the floor of the mouth (Howell et al. 1972).

Congenital epulis (Fuhr and Krogh 1972) is a benign soft tissue tumor that, on microscopy, shows a striking resemblance to a granular cell tumor. It usually arises from the alveolar mucosa of the premaxilla, and females are predominantly affected. It is usually single, but multiple lesions are described (Parmigiani et al. 2004). Some are large enough to cause respiratory obstruction (Bilen et al. 2004) or respiratory difficulties (Olson et al. 2005).

A *teratoma* arising from the basicranium, tonsillar area, or soft palate may protrude through the mouth (Lack 1985); it is usually benign but may obstruct the airway. There may be an intracranial extension in some cases (Smith et al. 1993). There may be interruption of normal palatal closure (Rotenberg et al. 2002).

The most common infection of the mouth in the newborn is moniliasis; there may be an associated maternal vulvovaginitis. It is also a frequent complication of antibiotic therapy in the neonate.

Salivary Glands

Malformations of the salivary glands are very uncommon. Absence of the parotid is rarely reported. A ranula is a retention cyst of the sub-

lingual or submaxillary gland ducts elevating the buccal epithelium. The cyst is usually unilocular and lined by mucus-secreting epithelium. Cytomegalovirus (CMV) infection often involves the salivary glands but is trivial compared with its effects on other organs. The most common tumor of the parotid in the neonate is a capillary hemangioma (Martinez-Mora et al. 1971). Rare neoplasms, designated choristoma (Tang et al. 1979), and embryoma (Vawter and Tefft 1966) have been described in the neonatal period.

Esophagus

Foci of ciliated pseudostratified columnar epithelium may persist within the esophagus and be identified in the neonatal period; they are more likely to be encountered in preterm babies. Gastric heterotopia may occur in the anterior subcricoid area of the esophagus (Variend and Howat 1988).

Mucosal erosions of the upper esophagus are a common histological finding among babies dying in the neonatal period (Merriam and Benirschke 1959). Only a proportion of cases can be explained on the basis of intubation.

Esophageal Atresia

Esophageal atresia may take one of several forms (Fig. 18.4A); the commonest is that where the upper esophagus ends as a blind pouch and the lower esophagus joins the respiratory tract through a fistula near the tracheal bifurcation (Fig. 18.4B) (Waterston et al. 1962). This variety accounts for over 80% of cases. Atresia of the esophagus without tracheoesophageal fistula is the next most common variety. Less common variants are shown in Fig. 18.4A. Associated anomalies are found in 48% of cases and commonly involve the cardiovascular system (Weaver et al. 1986) and gastrointestinal tract, including anal atresia (Holder et al. 1964). Mortality is adversely affected by low birth weight, which is probably related to the high incidence of polyhydramnios, and associated malformations. The latter were present in 61% of babies with esophageal atresia in a European tertiary referral center (Deurloo et al. 2004). Diaphragmatic hernia (Bochdalek) coexists more frequently than expected (van Dooren et al. 2005). Chromosomal abnormalities are found in 3% to 4% of neonates with esophageal atresia (Robertson et al. 1994).

Other anomalies that may rarely affect the esophagus are mucosal webs and diaphragms. Absence of the esophagus and double esophagus are rarely reported.

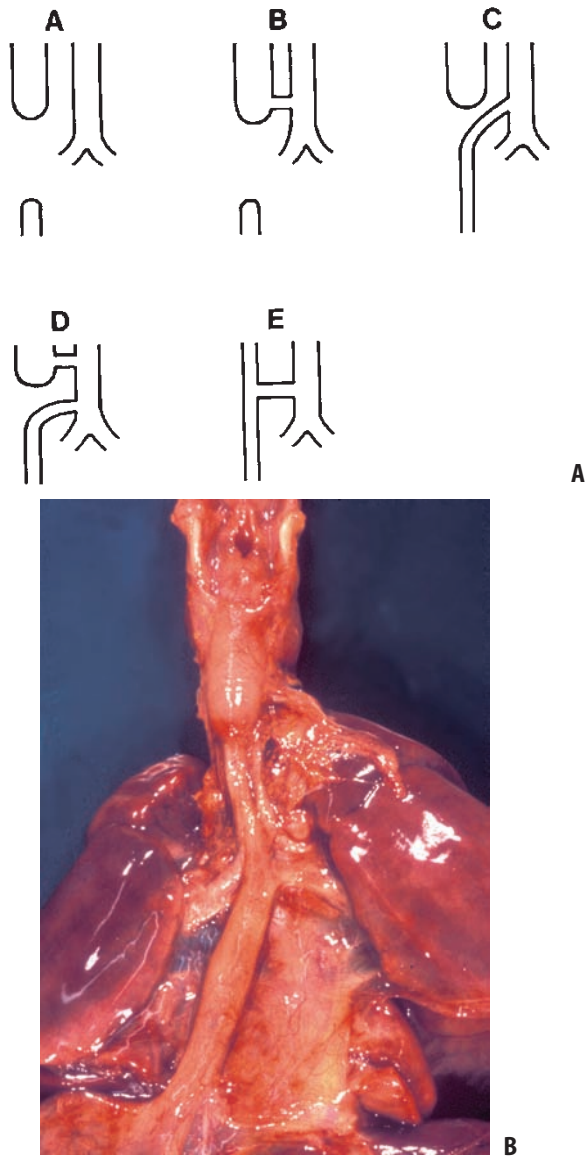


Figure 18.4. (A) Anatomical types of esophageal atresia and tracheoesophageal fistula. (B) Commonest type of defect. Thoracic viscera viewed from the back. The atretic upper pouch is thick walled and dilated.

The VATER association (Quan and Smith 1973; Temtamy and Miller 1974) is the nonrandom combination of vertebral anomalies (V), anal atresia (A), tracheoesophageal fistula (T-E) with atresia, renal defects (R), and radial limb dysplasia (R). A mesodermal defect operating in early development has been suggested as the basis for this association. A similar spectrum of malformations is found with trisomy 18. The acronym has more recently been changed to include the association with cardiac (C) and limb (L) abnormalities (VACTERL).

Stomach

Microgastria

Microgastria is a rare condition in which the stomach is considerably reduced in size and may retain a midsagittal position normally observed in the early embryo (Blank and Chisolm 1973). Asplenia may be associated. Esophageal atresia and diaphragmatic hernia are also described (Sharma and Menon 2005). Clinical presentation is usually related to feeding problems.

Infantile Hypertrophic Pyloric Stenosis

The incidence of pyloric stenosis in the United Kingdom is approximately 3 in 1000 live births. It is up to four times more common in boys than in girls, and familial cases are reported. This is the commonest condition requiring abdominal surgery in infancy. Vomiting is the commonest presenting symptom and usually starts after the first week of life. Because of the excellent prognosis that follows current management, the pathologist is unlikely to encounter this abnormality as a cause of upper intestinal obstruction. The pathogenesis remains unclear. Altered expression of neuronal nitric oxide synthetase (Saur et al. 2004) and hemeoxygenase-2 (Piotrowska et al. 2003) may contribute to the dysmotility. Elevated somatostatin but low gastrin levels are described at the time of pyloromyotomy (Dick et al. 2001). Early exposure to erythromycin increases the risk up to eightfold (Cooper et al. 2002). A polygenic mode of inheritance is most likely (Carter and Evans 1969).

The pylorus is increased in length and external diameter, the stomach is dilated, and the antrum is hypertrophic. The circular muscle layer is up to four times the normal thickness and the longitudinal muscle is attenuated. Evidence of previous pyloromyotomy is sometimes seen in infants dying from other causes. A longitudinal brown line about 1.5 cm in length is visible in the anterior wall of the pylorus. Residual myohypertrophy may be present.

Spontaneous Gastric Perforation

The predominant symptom of spontaneous gastric perforation is the sudden onset of abdominal distention during the first week; there is a high incidence of prematurity. The majority of perforations occur high on the greater curvature (Parrish et al. 1964). Theories of pathogenesis include tissue ischemia secondary to hypoxia (Touloukian 1973), mechanical disruption, stress ulcers, and perforations from feeding catheters (Holgersen 1981). Gastric perforation related to distal obstruction is generally not included in this category.

Peptic Ulceration

Gastric and duodenal ulcers are both unusual in the neonatal period; duodenal ulcers are slightly more common (Nacheff et al. 1964; Bell et al. 1981). No single etiological factor seems to be responsible. Perforation or hemorrhage, or both, are the usual clinical presentations. Of all cases reported in children, peptic ulcer in the neonate accounts for approximately 10%. Peptic ulcers in the newborn are always acute, and microscopy shows little evidence of inflammatory reaction. Neonatal gastric ulceration may be iatrogenic; it is a reported complication of tolazoline administration (see Fig. 17.17 in Chapter 17). Mucosal erosions of the neonatal stomach are far more common and are usually seen in association with severe asphyxia or overwhelming sepsis. Hemorrhage from deep ulcers may be severe and life-threatening.

Pyloric Atresia

Pyloric atresia is reported to occur in one per million live births (Robertson et al. 1994). In

common with most intestinal atresias, it is probably secondary to in utero vascular compromise. Polyhydramnios is common and the condition is diagnosable in utero on ultrasound examination. Epidermolysis bullosa, a rare autosomal recessive disease that is often fatal, may be associated (Chang et al. 1983).

Small Intestine

Enteric Duplication and Mesenteric Cysts

Intestinal duplication or enterogenous cysts are spherical or tubular structures possessing the basic pattern of the enteric wall. Any part of the alimentary tract may be affected, but duplications are usually intraabdominal. The small intestine is most commonly involved, particularly the distal ileum (Dohn and Povlsen 1951; Grosfeld et al. 1970). Of the foregut duplications, those associated with the esophagus are most common (Bissler and Klein 1988); gastric duplications are usually located on the greater curvature and are twice as common in females, and can produce gastric outlet obstruction (Master et al. 2004).

A variety of mechanisms have been invoked to explain the origin of duplication cysts. These include failure of recanalization of the bowel lumen following the so-called solid epithelial phase of intestinal development (Bremer 1944), persistence of epithelial outpouchings described in embryonic intestine (Bremer 1944), and intestinal ischemia in early intrauterine life (Favara et al. 1971). A mechanism that is currently popular implicates impaired separation of the notochord from intestinal endoderm (Veeneklaas 1952; Fallon et al. 1954) and the formation of neurenteric bands that, with embryonic growth, produce traction diverticula. This has been demonstrated experimentally in amphibian embryos (Emura et al. 2003). It has been suggested that enteric duplication, congenital intestinal diverticulum, and segmental dilatation of the intestine are pathogenetically related (Heller et al. 1989).

Foregut duplications often cause respiratory distress while those of the mid- and hindgut are associated with a palpable mass, or intestinal obstruction caused by direct mechanical pressure (Grosfeld et al. 1970). Rectal duplications are rare and may present as a sacrococcygeal mass (Lemire et al. 1971). Intussusception occasionally occurs (Cunningham et al. 1980). Those cysts containing gastric mucosa (Fig. 18.5) are vulnerable to ulceration, accompanied by perforation and hemorrhage. Heterotopic tissue found in duplications are probably derived from the primitive gut.

Most duplication cysts are located close to the mesenteric border of the intestine (Fig. 18.5), often lying within the mesentery and sharing a common blood supply, or are situated within the muscularis propria. Occasionally, they are more

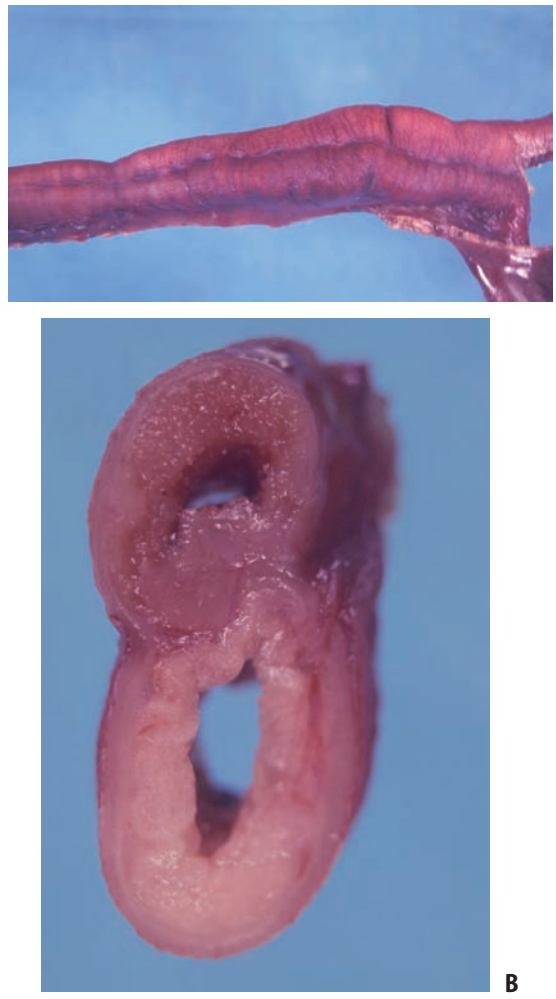


Figure 18.5. Ileal duplication. (A) Tubular duplication is closely applied to the mesenteric border of the intestine. (B) Transverse section through the resected specimen. The duplication is lined by gastric mucosa, paler than the intestinal lining. (From Courtesy Dr. K.J. McKenzie, Edinburgh, Scotland.)

widely separated from the intestine and lie in the retroperitoneum (Duncan et al. 1992). Cysts are usually single and exceptionally communicate with the bowel lumen. About 20% of duplications are multiple.

A smooth muscle coat, which sometimes contains a neural plexus, is always present. The mucosal lining may be of the contiguous parent intestine or resemble that of a more distant site. Many duplication cysts, irrespective of location, contain gastric mucosa.

Sometimes duplications or their fibrous connections pass through the diaphragm, producing bizarre symptoms (McLetchie et al. 1954; Goldberg and Johnson 1962). Some duplication cysts, particularly those in the mediastinum, may be attached directly or via a fibrous cord to a spinal defect of some sort; vertical clefts, hemivertebrae, and anterior spina bifida are all described. The lower cervical and upper thoracic vertebrae are usually involved. Mediastinal and abdominal cysts may coexist (Fallon et al. 1954). Associated congenital abnormalities occur in some 20% of affected infants. Alimentary duplication and pulmonary sequestration may coexist as part of a bronchopulmonary foregut malformation complex (Brink and Balsara 1991). Some contain heterotopic pancreatic tissue (de Krijger et al. 2004).

Mesenteric cysts are rare, the majority being lymphatic or mesothelial (De Perrot et al. 2000). Presentation in the neonatal period is unusual.

Split Notochord Syndrome

Split notochord syndrome comprises a spectrum of rare anomalies, ranging from dorsal cysts to bowel herniating between incomplete vertebral arches. Cleft vertebral bodies and vertebral arch defects are invariably present (Bentley and Smith 1960; Smith 1960). Related malformations have been described by Esterly and Baghdassarian (1963) and Denes et al. (1967). It has been suggested that a common pathogenetic mechanism involves localized division of the notochord and persistence of connections between notochord and endoderm. These abnormalities may be part of a spectrum that embraces duplication cysts, intestinal diverticulae, and neurenteric fistulas. Prenatal diagnosis in the presence of polyhydramnios is described (Almog et al. 2001).

Vitellointestinal Duct Remnants

A spectrum of anomalies relate to persistence of part or all of the vitellointestinal duct (Smith 1960). Meckel's diverticulum is the commonest congenital anomaly of the gastrointestinal tract and is present in 2% of the population; boys are more commonly affected. It occurs with greater frequency in infants with trisomy 13 and 18 and results from failure of obliteration of the intestinal end of the duct.

The diverticulum is sited on the antimesenteric border of the terminal ileum and is usually of the same diameter as the intestine from which it arises. It is lined by small intestinal mucosa, but pancreatic and gastric heterotopias are often present. Perforation, hemorrhage, volvulus, and intussusception are recognized complications but rarely occur in the neonatal period.

A fibrous cord may connect the ileum or a Meckel's diverticulum to the abdominal wall at the umbilicus. It may cause volvulus and intestinal obstruction (Fig. 18.6). A cyst may occur at any point along the course of the original vitelline duct. Persistence of intestinal mucosa at the umbilicus (vitelline sinus, enterocystoma) may require excision because of mucus secretion or hemorrhage (Fig. 18.7).

Exceptionally, the entire vitellointestinal duct remains patent, and meconium leaks from the umbilicus shortly after birth (Jackson and Bird 1961). Ileal intussusception through a patent vitellointestinal duct is rare; everted small bowel may mimic a ruptured exomphalos or gastroschisis (Moore and Schumacker 1952).

Abnormalities of Intestinal Rotation and Fixation

Failure of the embryonic intestine to undergo rotation during and after its return to the abdominal cavity may be complete. More commonly, however, the initial counterclockwise 180-degree rotation takes place, but the final stage of rotation may arrest at that time or any stage before the cecum has descended to the right iliac fossa (Fig. 18.8).

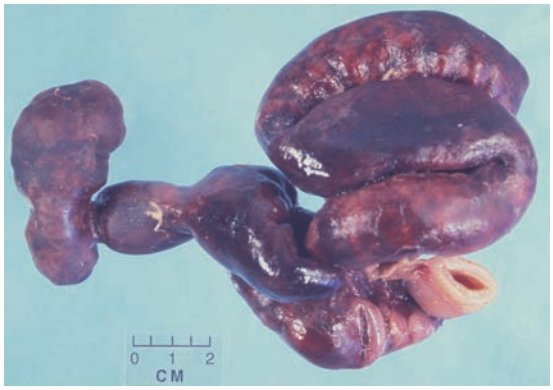


FIGURE 18.6. Volvulus complicating Meckel's diverticulum and persistent vitellointestinal duct.

When intestinal rotation does not occur (nonrotation), the entire colon is on the left side of the abdomen with the small bowel lying on the right. This is the pattern usually associated with diaphragmatic hernia and exomphalos but may be an incidental finding at necropsy.

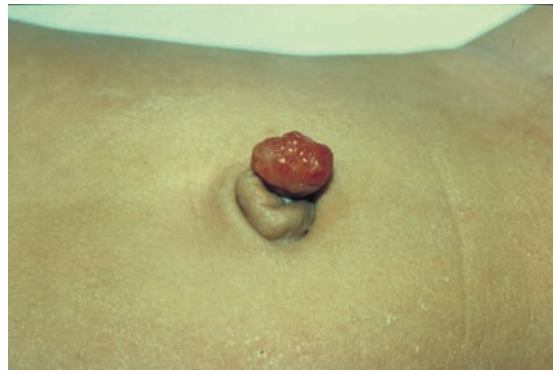
When intestinal rotation is incomplete (malrotation), the cecum may lie in the upper central abdomen anterior to the duodenojejunal junction (Snyder and Chaffin 1954). The mesenteric attachment of the midgut is short, rendering the intestine unduly mobile, and midgut volvulus is a frequent complication. This may present in the neonatal period as intestinal obstruction and gangrene, or may result in small intestinal atresia or meconium peritonitis if volvulus occurs in utero. Around 13% of cases of jejunoileal atresia are associated with malrotation (Robertson et al. 1994).

When cecal descent is incomplete, the peritoneal bands that normally run between the right peritoneal gutter and right colon after resorption of the right mesocolon and affect colonic fixation are abnormal in length and situation. They persist as Ladd's bands, which cross anterior to the duodenum and may cause extrinsic obstruction (Stewart et al. 1976).

Rarely, reversed rotation occurs when the intestine rotates in a clockwise direction rather. The superior mesenteric artery passes anterior to, and may obstruct, the transverse colon, with the duodenum lying anterior to the artery (Kiesewetter and Smith 1958).

Malrotation is prevalent in trisomy 9, 13, 18, and 21, and is also reported in association with triploidy and the cri-du-chat syndrome. The condition affects approximately 1 in 12,000 live births (Robertson et al. 1994).

Heterotaxia (or partial situs inversus) refers to an abnormal arrangement of body organs that is neither complete situs solitus nor complete situs inversus (Chang et al. 1993). Intestinal rotational abnormalities, as well as cardiac defects, are frequently associated.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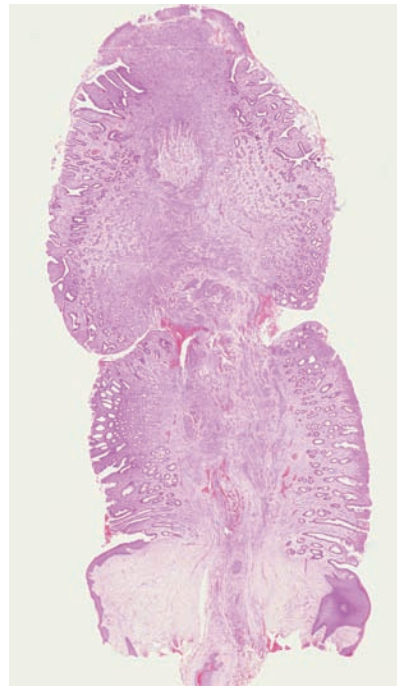
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FIGURE 18.7. Vitellointestinal remnant. (A) Mucosal-covered nodule at the umbilicus. (B) Section through a whole polyp. It is covered by mucosa, there is ulceration at the tip.

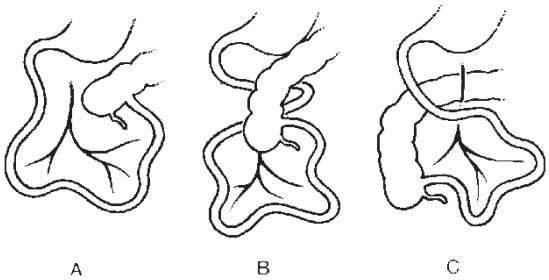


Figure 18.8. Different patterns of rotational abnormality of the intestine. (A) Nonrotation. (B) Malrotation. (C) Reversed rotation.

Congenital Short Intestine

Short small bowel has been described in association with exomphalos (Reiquam et al. 1965) and gastroschisis, conditions known to be associated with prenatal volvulus. Bowel infarction, resorption of the infarcted bowel, and spontaneous reanastomosis of the lumen has been suggested as the underlying cause. A similar mechanism has been proposed for reduced intestinal length in babies with jejunal or ileal atresia (Benson et al. 1960). Congenital short intestine is occasionally reported as an isolated defect (Yutani et al. 1973). Familial cases are described (Erez et al. 2001).

Intestinal Atresia and Stenosis

Atresia is a loss of continuity of the bowel lumen and is accompanied by intestinal obstruction, while stenosis is a localized luminal narrowing that may produce a partial obstruction to passage of intestinal contents. The duodenum is most commonly affected, its incidence being about 1 in 5000 live births. Involvement of the jejunum and ileum are less common, while atresias of the colon are rare. Antral mucosal web is a rare cause of gastric outlet obstruction (Bell et al. 1978); there is a high incidence of associated congenital anomalies. Its relationship to atresia occurring at other levels of the intestine is unclear.

Presenting symptoms are vomiting, abdominal distention, and failure to pass meconium. There is a recognized association between atresia and maternal polyhydramnios, especially with proximal bowel involvement.

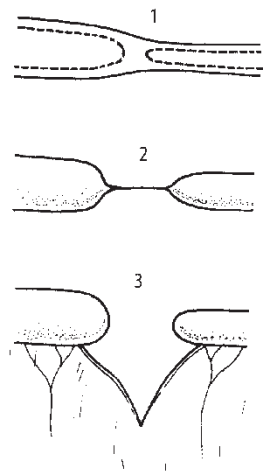


Figure 18.9. Different types of intestinal atresia.

Three forms of atresia are recognized (Santulli and Blanc 1961) (Fig. 18.9):

1. The muscularis propria is uninterrupted, and a diaphragm composed of connective tissue covered on both sides by mucosa separates the dilated proximal bowel from the distal collapsed segment. Sometimes the occluding diaphragm has a central perforation.
2. There is loss of continuity of the intestine, and the proximal dilated segment is separated from the distal intestine by a fibrous cord (Fig. 18.10).
3. There is a gap between the proximal and distal segments and a wedge-shaped defect in the mesentery is frequently present.

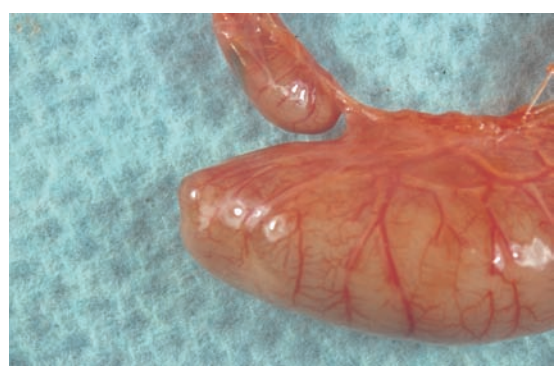


Figure 18.10. Type 2(B) intestinal atresia.

Atresias are multiple in 15% of cases, and two or more of the three types described may coexist in the same patient. Type 3 is the most common.

Duodenal Atresia

Duodenal atresia or stenosis occurs in approximately 1 in 10,000 live births (Robertson et al. 1994). Atresia is far more common than stenosis. The type 1 pattern is the most common duodenal abnormality. Extraintestinal anomalies are more commonly associated with duodenal atresia than with distal intestinal atresias, and it is strongly associated with Down syndrome (Young and Wilkinson 1968; Fonkalsrud et al. 1969). The associated defects are usually cardiac malformations; anorectal anomalies are also described. Duodenal atresia is accompanied by polyhydramnios in 17% to 35% of cases, often leading to premature labour (Robertson et al. 1994). It is found in Feingold syndrome, a dominantly inherited disorder comprising esophageal atresia/tracheoesophageal atresia, microcephaly, facial dysmorphism, hand/foot anomalies, and developmental delay (Holder-Espinasse et al. 2004).

Duodenal atresia, unlike more distal intestinal atresias, appears to result from defective embryogenesis. Failure of recanalization of the intestinal lumen following florid epithelial proliferation about the 8th week of gestation is the suggested mechanism (Tandler 1900, cited by Santulli and Blanc 1961). The duodenum is the only point in the intestine where epithelial proliferation completely occludes the lumen (Fig. 18.11). Duodenal atresia is often accompanied by annular pancreas, further supporting the concept of locally defective embryogenesis. Annular pancreas can produce extrinsic compression or intrinsic obstruction due to accompanying atresia or stenosis. The duodenal obstruction may be diagnosed prenatally by ultrasonography, which shows the characteristic "double-bubble" sign (Robertson et al. 1994).

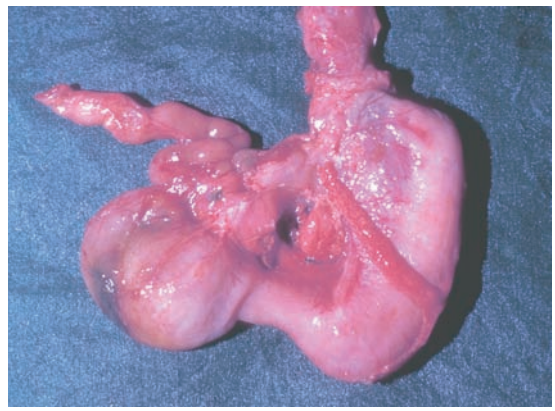


Figure 18.11. Duodenal atresia; the stomach is slightly dilated and is separated from the dilated duodenum by the normal pyloric constriction.

Jejunal and Ileal Atresia

Previously thought to follow failure of recanalization of the solid stage (see above), atresia and stenosis have been produced experimentally in animals by occlusion of branches of the superior mesenteric artery, thus rendering the intestine ischemic in utero (Louw 1966; Tibboel et al. 1980). The presence of fetal squamae and lanugo hairs in the lumen distal to the atresia provides evidence that the atresia develops after patency of the bowel lumen and peristalsis have become established (Santulli and Blanc 1961). Evidence of fetal bowel infarction at operation was found in 42% of cases of jejunoileal atresia by deLorimier et al. (1969), and peritonitis was observed in 48% of jejunoileal atresias by Nixon and Tawes (1971). When associated anomalies occur in jejunoileal atresia, they generally involve the gastrointestinal tract; malrotation of the intestine, volvulus, gastroschisis, intussusception, and umbilical attachment are described. Jejunoileal atresias or stenosis may accompany meconium ileus, and an association with amyoplasia congenita has also been reported (Collins et al. 1986).

The bowel proximal to the atresia is always bulbous and its vascularity may be seriously impaired; perforation is a recognized complication. The distal bowel is contracted with a narrow lumen.

A rare variant of intestinal atresia is the "Christmas tree," "maypole," or "apple peel" deformity (Fig. 18.12) (Weitzman and Vanderhoof 1966; Dickson 1970). The lesion comprises a duodenal or high jejunal atresia and absence of the dorsal mesentery. The distal small bowel is considerably reduced in length and it is twisted round a

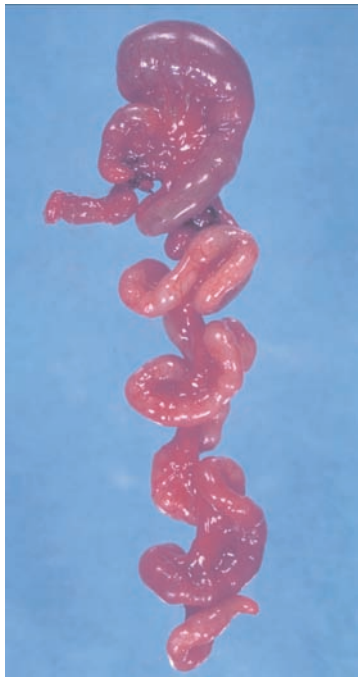


Figure 18.12. "Apple peel" variant of intestinal atresia. The proximal jejunum is dilated (top), and the adjacent bowel is coiled round the marginal artery.

marginal artery. This variety of intestinal atresia has been reported in families (Blyth and Dickson 1969). A similar constellation of anomalies, also familial, is distinguished by its authors from apple-peel deformity (Pumberger et al. 2002).

Colonic Atresia

Colonic atresia accounts for less than 10% of intestinal atresias (Robertson et al. 1994), the majority occurring proximal to the splenic flexure. The mechanism is thought to be in utero vascular compromise, similar to jejunoileal atresias.

Meconium Abnormalities

Meconium Ileus

Meconium ileus is the earliest manifestation of cystic fibrosis (mucoviscidosis). Its incidence is reported in between 7% and 25% of affected individuals. The majority of affected babies are born at term. Intestinal obstruction associated with failure to pass meconium is apparent in the first few hours or days after birth and is always present from birth. Babies who survive the neonatal period eventually develop other signs and symptoms of mucoviscidosis, which are largely related to malabsorption and recurrent respiratory infection. Maternal polyhydramnios is common (Holsclaw et al. 1965).

Radiological examination of the abdomen shows a mottled granular or ground-glass appearance and a characteristic paucity of fluid levels (Kopel 1972). Intestinal obstruction is due to an intraluminal bolus of abnormally viscid meconium. There is a dilated loop of bowel, often rigid because of impacted meconium (Fig. 18.13), which, when removed, forms a cast of the bowel lumen. The more proximal bowel is dilated and its wall may be hypertrophied; due to the action of intestinal bacteria after birth, the content at this level is usually semifluid. The colon is very small,

Figure 18.13. Meconium ileus. (A) Dilated loop of bowel filled with inspissated meconium. The serosa is congested. (B) Opened bowel reveals a mass of gray-green meconium.

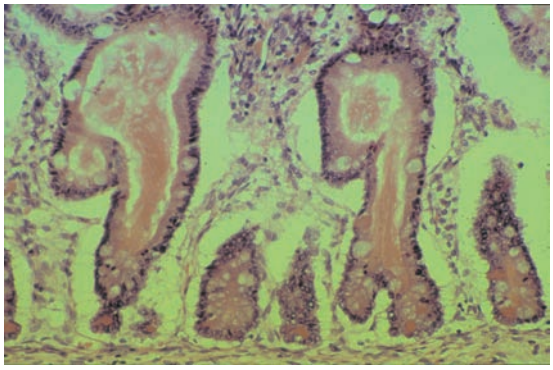


Figure 18.14. Postmortem sample of small intestine from a preterm baby. There is acidophilic secretion within dilated crypts.

and, because of failure of significant passage of meconium beyond the level of obstruction, tiny concretions of gray inspissated meconium with a consistency of dried putty are scattered along the colon.

Meconium ileus can be diagnosed on ultrasound examination in utero as early as 17 to 19 weeks' gestation (Boué et al. 1986), with hyperechogenic bowel in the right lower abdominal quadrant. In most cases, the picture resolves later in gestation; when it persists, meconium ileus presents at birth. Fetal meconium ileus can be confirmed at autopsy; there is a narrow empty colon, variable lengths of dilated ileum with a beaded appearance due to white or gray pellets of inspissated meconium, and dilated bowel proximally (Muller et al. 1985; Boué et al. 1986). Microscopically, periodic acid–Schiff (PAS)-positive amorphous material may be detected within the pancreatic acini, but the intra- and extralobular ducts are normal. These appearances are nonspecific.

In the neonate, mucosal glands of the small intestine contain masses of acidophil secretion (Fig. 18.14) and may be cystically dilated. Brunner's glands may be similarly dilated by "stringy" mucus. Two factors explain the consistency of the meconium: pancreatic insufficiency resulting in deficient protein digestion; and abnormal viscid tenacious mucus produced by the intestinal goblet cells, which is not released into the lumen in normal amounts. As the pancreas in the neonate may be normal or show minimal abnormality, it is thought that abnormality of the intestinal mucous glands is more important (Thomaidis and Arey 1963). However, partial pancreatic aplasia and stenosis of the pancreatic duct are reported in meconium ileus (Park and Grand 1981).

The incidence of associated nonintestinal abnormalities is no different from that in the general population (Holsclaw et al. 1965; Donnison et al. 1966). Atresia, stenosis, volvulus, peritoneal bands, gangrene, and perforation are fairly frequent, and are considered secondary mechanical complications (Oppenheimer and Esterly 1962). Intestinal atresia and stenosis in meconium ileus may be the result of localized pressure on the intestinal wall from inspissated meconium producing ulceration, granuloma formation and scarring, or prenatal volvulus of meconium-loaded loops of small bowel (Bernstein et al. 1960).

The incidence of cystic fibrosis among patients with intestinal atresia is about 10% (Nixon and Tawes 1971), but cystic fibrosis should be excluded in all cases of atresia of the distal bowel as the prognosis is much worse and the implications for future pregnancies very serious.

Meconium Plug Syndrome

Transient obstruction caused by sticky meconium involving distal colon and rectum has been termed "meconium plug syndrome" (Clatworthy et al. 1956) and is the mildest form of meconium obstruction in the newborn. Clinical presentation is with mild abdominal distention, bilious vomiting, and failure to pass meconium within the first 24 hours of life (Olsen et al. 1982). The condition may settle with the spontaneous passage of meconium or following an enema. Burge and Drewett (2004) draw attention to the clinical and radiological overlap with small left colon syndrome. They found a higher incidence of Hirschsprung's disease (HD) among babies presenting with meconium plug obstruction than previously reported, and recommend exclusion of both HD and cystic fibrosis in all cases.

Meconium Disease (or Inspissated Meconium Syndrome)

Intestinal obstruction caused by plugs of sticky meconium blocking the distal small bowel and

proximal colon in the neonate was first reported in the absence of cystic fibrosis by Rickham and Boeckman (1965). Vinograd et al. (1983) reported seven cases in infants of very low birth weight with normal sweat tests. These cases differ from meconium ileus both radiologically and because intestinal obstruction is not present from birth. Garza-Cox et al. (2004) found a strong association between neonatal obstruction of the gastrointestinal tract by meconium, fetal growth restriction, and maternal hypertensive disorders. They suggest a role for reduced intestinal perfusion in utero.

A similar clinical presentation was reported by King et al. (1986) in six preterm babies with respiratory problems and sepsis who passed meconium in the first 3 days of life but subsequently exhibited intestinal obstruction. At necropsy the small intestine was distended by abnormal meconium. The colon was small; inspissated secretions were present in gland crypts. Within the pancreas, acinar and ductular dilatation, focal acinar atrophy, and patchy fibrosis were apparent. Similar changes were present in salivary glands of some babies. Immunoreactive trypsin levels were normal in all six babies. These authors suggested that fluid restriction, uremia, and sepsis may be important in the development of this condition and questioned the specificity of the necropsy findings in meconium ileus.

Pseudomeconium ileus is a term that has been applied to a form of ileus diagnosed in utero, unassociated with inspissated meconium, and attributed to cytomegalo-inclusion disease involving the enteric ganglia (Déchelotte et al. 1992). Vasculitis of the intestinal wall may explain the neonatal intestinal obstruction seen with congenital syphilis (Siplovich et al. 1988).

A transient disturbance of colonic motility, designated *small left colon syndrome*, has been reported in infants of diabetic mothers (Philippart et al. 1975). Blockage of the distal small bowel caused by impacted milk curd is occasionally reported (Cook and Rickham 1969). Clinical obstruction is usually manifest during the second week after birth. The condition appears to be related to early high-calorie formula feeds. It is not associated with cystic fibrosis and ganglion cells appear normal.

Meconium Peritonitis

Meconium peritonitis is a sterile chemical peritonitis following prenatal or perinatal perforation of the bowel, usually associated with intestinal obstruction (Rickham 1955; White 1956). Perforation, which most commonly involves the ileum, occurs at any time from the 5th month of fetal life until bacterial colonization of the intestine at birth. Peristalsis, which is necessary to extrude meconium, rarely occurs before the 20th week of gestation (Konje et al. 1995). In the majority of cases, the perforation has closed by the time of birth. Abdominal distention may be present at birth or develop during the first 24 hours. Cystic fibrosis is reported in 7.7% to 50% of patients with meconium peritonitis (Konje et al. 1995).

Meconium peritonitis may be diagnosed on ultrasound examination as early as the 30th week of gestation (Konje et al. 1995; Eckoldt et al. 2003). The demonstration of intraabdominal calcification in the fetus on ultrasonography is virtually pathognomonic of the condition, and calcification within the bowel lumen or wall and involvement of peritoneal surfaces is seen in a high proportion of cases. Exceptions are calcification involving the intestinal content (enterolithiasis) seen in newborn babies with anorectal malformation with rectourinary fistula (Berdon et al. 1975; Miller et al. 1988) and hydrometrocolpos (Hu and Methratta 2001).

At birth the most useful investigation in meconium peritonitis is a plain abdominal radiograph, which reveals an opaque ground-glass appearance speckled with calcification. Radiological demonstration of calcification in the scrotum is considered pathognomonic and is due to the passage of meconium and exudate down a patent processus vaginalis (Fig. 18.15); hard calcified nodules may develop in the scrotum or inguinal region subsequently (Forouhar 1982).

The pathology of the condition depends largely on the time at which perforation occurs and may be classified as fibroadhesive, cystic, or generalized (Lorimer and Ellis 1966). In the fibroadhesive form, probably related to early prenatal perforation, a fibroplastic reaction seals off the intestine at the site of the perforation. Calcification is common (Fig. 18.16) and perforation may not be detected at operation or postmortem. In the cystic

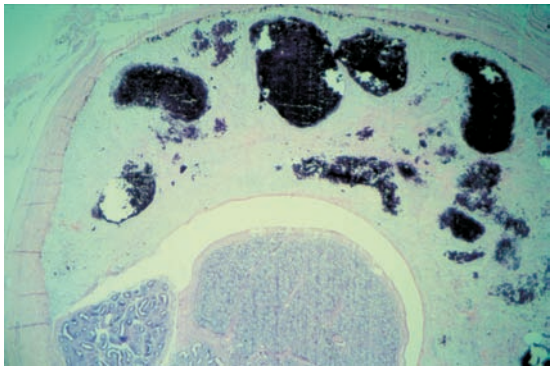


Figure 18.15. Exudate with patchy calcification around the testis in an infant with meconium peritonitis.

type, a thick-walled cyst, isolated from the remainder of the abdominal viscera, forms around the perforation. The cyst is filled with exudate and its lining is calcified.

When perforation occurs in the perinatal period, a generalized form of peritonitis develops, with meconium-stained viscid exudate distributed widely throughout the peritoneal cavity.

Jejunoileal atresia and meconium ileus, singly or combined, account for the majority of cases (Smith and Clatworthy 1961). Meconium peritonitis unassociated with intestinal obstruction may follow episodes of intestinal ischemia in utero leading to bowel wall damage, disruption, and repair (Tibboel et al. 1986).

Meconium peritonitis complicating cystic fibrosis carries a poor prognosis. In the absence of cystic fibrosis, dilated loops of bowel, meconium pseudocyst, ascites, or polyhydramnios as determined on ultrasonography characterize complex meconium peritonitis, also associated with a poor outlook (Dirkes et al. 1995).

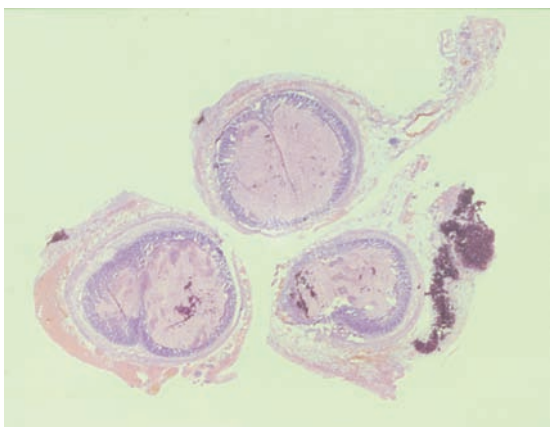


Figure 18.16. Small intestine in meconium peritonitis. There is thickening of the serosa with focal calcification.

Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is increasingly common, affecting small preterm infants, in particular those weighing less than 2000 g (Dudgeon et al. 1973), although mature babies are also affected (Rodin et al. 1973). It is usually apparent during the first 2 weeks of life, but may be seen later in preterm babies. The increasing incidence was partly explained by survival of very small, preterm babies. However, better management of respiratory problems in the postsurfactant era has seen a reduction in the number of cases and polarization of risk factors at different gestations (Luig and Lui 2005). Survival among extremely low birth weight infants remains poor (Blakely et al. 2005). Pallor, lethargy, vomiting, abdominal distention, diarrhea, and blood-stained stools are the usual clinical features.

Pneumatosis intestinalis is the radiological hallmark of the condition (Mizrahi et al. 1965) (see Fig. 11.6 in Chapter 11), but intestinal dilatation may be the only abnormality. Pneumoperitoneum indicates intestinal perforation, a frequent complication. Gas is seen occasionally in the portal vein or liver (Dudgeon et al. 1973) (Fig. 18.17). Appendicitis in the neonate is almost always due to necrotizing enterocolitis (Santulli et al. 1975; Bax et al. 1980).

Pneumatosis may result from intraluminal gas diffusing through ischemic mucosa or may be liberated by organisms colonizing devitalized tissue (Stevenson et al. 1969). Both intramural and intraluminal gas from affected neonates comprise more than 30% hydrogen (Engel et al. 1973).

The pathogenesis of NEC is still incompletely understood (Kliegman 1990) and has been reviewed by Hsueh et al. (1998, 2002). Likely factors include an altered mucosal integrity, oral feeding, and bacterial colonization of the bowel lumen. A high incidence of perinatal asphyxia has been reported in one series (Lloyd 1969). Hypoxia–ischemia is less favored nowadays and there is increasing evidence that the compromised

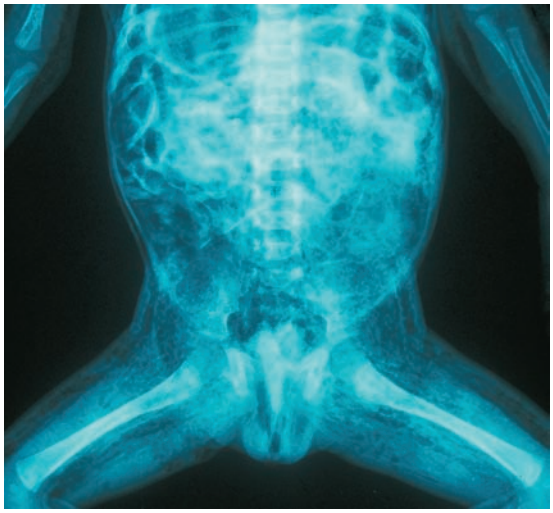


Figure 18.17. Necrotizing enterocolitis. There is pneumoperitoneum and extensive intravascular gas.

mucosal integrity may be related to prematurity itself.

It has long been recognized that NEC does not occur in a germ-free environment (Faix and Adams 1994). A wide variety of bacteria has been isolated from affected infants, and severity of disease may be related to the type of organism (Howard et al. 1977; Faix and Adams 1994). Microorganisms play a critical role in this disease, but their role is probably secondary. Probiotics, both indigenous (Hsueh et al. 2002) and administered (Lin et al. 2005), reduce both the incidence and severity of NEC in very low birth weight babies. Coutinho et al. (1998) draw attention to the absence of lysozyme in small intestinal Paneth cells in this condition as a predisposing factor for infection.

Necrotizing enterocolitis can occur prior to oral feeding (Marchildon et al. 1982). These infants were generally of lower birth weight and maturity and had a higher incidence of perinatal asphyxia and respiratory distress syndrome than those who developed NEC after feeding. A proportion of these infants also had pneumatosis intestinalis. The development of NEC in the absence of feeding suggests that compromised mucosal integrity has a more significant role. Factors that reduce enterocyte apoptosis such as the glutathione antioxidant system (Kelly et al. 2004) and epidermal growth factor (Clark et al. 2005) protect against NEC in the experimental animal.

Systemic stress causes a breakdown in the intestinal mucosal barrier with increased enterocyte apoptosis and decreased enterocyte migration (Hackam et al. 2005). Vieten et al. (2005) suggest a role for trefoil factor peptides that promote epithelial repair and restitution. They found downregulation of their expression in 83% of surgical specimens from NEC patients. Oxygen-derived free radicals, platelet-activating factor, and tumor necrosis factor-α, local factors that induce local tissue necrosis, have a role in tissue necrosis and repair (Antonioli 1997). Hsueh et al. (2002) stress the importance of platelet-activating factor in both the initiation and extension of mucosal injury in NEC. Inflammation may also be enhanced by macrophage migration-inhibitory factor (Ren et al. 2005).

Aminophylline, a xanthine derivative, is often employed as a stimulant in the management of neonates with episodes of apnea. Aminophylline decreases gastrointestinal motility and promotes intraluminal bacterial overgrowth. Its role as a factor in some cases of NEC has been questioned (Grosfeld et al. 1983).

Necrotizing enterocolitis has been described following exchange transfusion (Corkery et al. 1968), umbilical arterial catheterization (Franz et al. 1975), cardiac catheterization (Bunton et al. 1977), and hyperviscosity (Hakanson and Oh 1977). A high mortality is associated with NEC following umbilical arterial catheterization, whereas the mortality following exchange transfusion is quite low (Antonioli 1997).

The terminal ileum and proximal colon are affected most commonly (Fig. 18.18), but more

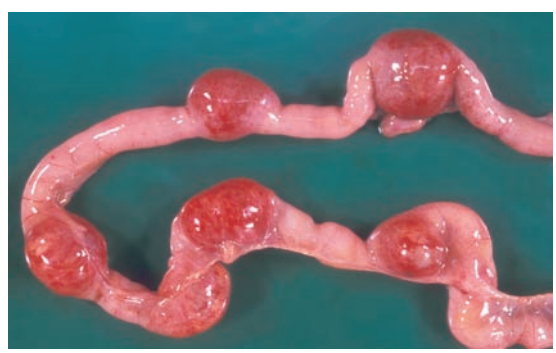


Figure 18.18. Necrotizing enterocolitis. The small intestine from a preterm twin. Well-circumscribed, suffused, dilated areas in the bowel wall indicate points where the muscularis propria is damaged.

proximal or distal gut may be involved. Rarely, the entire intestinal tract below the duodenum is affected. The bowel wall may be thickened and congested and later becomes thin and friable. Perforation, which may be single or multiple, often occurs along the antimesenteric border. Gas blebs may be visible beneath the serosa. Histological appearances are related to the stage and severity of the disease. Initially, edema, hemorrhage, coagulation necrosis, inflammation, and pneumatosis predominate (Fig. 18.19). Pseudomembrane formation associated with mucosal necrosis (without preceding antibiotic treatment) may be prominent. These changes may be limited to the mucosa or extend deeper to involve submucosa and muscle. Pneumatosis may involve all layers of the intestinal wall but frequently accumulates in the loose connective tissue of the submucosa. Ganglion cells, being more resistant to hypoxia–ischemia, are generally spared. Localized perforation of the bowel wall with little or no reaction in the adjacent tissue has been described (Lloyd 1969; Marchildon et al. 1982). Mortality in the acute phase is related to perforation, septicemia, and systemic collapse.

Epithelial regeneration and granulation characterize the repair phase, and residual damage in the form of fibrosis and mural thickening frequently results in luminal obstruction (Fig. 18.20). The majority of strictures are found in the colon, usually the left side (Faix and Adams 1994). The ileum is the most frequent site for small bowel stricture. Other late complications, secondary to extensive resection of necrotic bowel, include short-gut syndrome.

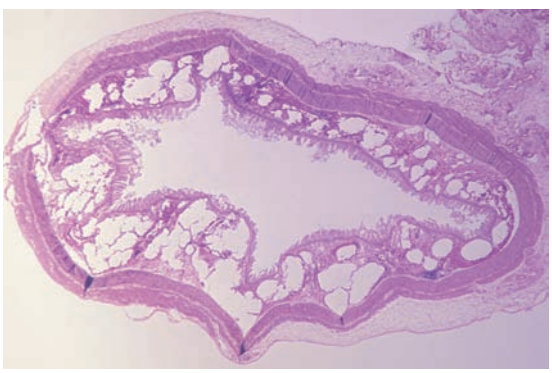


Figure 18.19. Pneumatosis intestinalis. Gas bubbles distort submucosal anatomy.

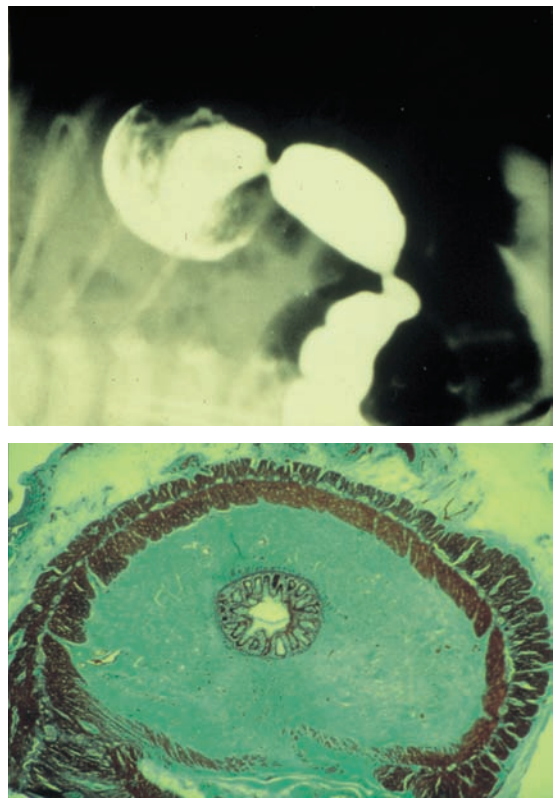


Figure 18.20. Necrotizing enterocolitis—late complication. (A) Barium enema shows two strictures in the descending colon. (B) Marked luminal narrowing with submucosal fibrosis is encountered when the muscularis mucosae is damaged in the acute stage.

Bowel Ischemia Associated with Thromboembolic Disease

Extensive infarction of the small bowel and colon may occur as a result of thrombosis of the superior mesenteric artery (Rothschild et al. 1953) or aorta. The origin of the thrombus may not be apparent or may follow umbilical arterial catheterization (Cochran et al. 1968; Neal et al. 1972) (see Fig. 17.13 in Chapter 17).

Colon and Rectum

Hirschsprung's Disease

Hirschsprung's disease (HD) is a disorder characterized by aganglionosis of the distal rectum with involvement of the immediately proximal bowel

to a variable extent. Aganglionosis interrupts peristalsis, leading to intestinal obstruction.

Hirschsprung's disease affects about 1 in 5000 liveborns, although estimates of 1 in 1000 to 1 in 30,000 liveborns are quoted (Lister and Rickham 1980). Males are more commonly affected (about 3:1 to 5:1). The condition accounts for approximately 15% of all cases of neonatal intestinal obstruction (Santulli and Amoury 1967). Around 8% of patients with HD have a positive family history; this is more common when a long segment of colon is involved (Engum et al. 1993). Dominant and recessive pattern of inheritance and the increased risk in siblings and in Down syndrome point to the importance of genetic factors in pathogenesis of the condition. Kapur (1999a) opines that most, if not all, cases of HD have a genetic basis. Mutations in the receptor tyrosine kinase *ret* gene on chromosome 10q11.1 and the endothelin-B receptor gene on chromosome 13q22 (Sullivan 1996; Qualman et al. 1997), as well as the transcription factors Sox10 (Parisi and Kapur 2000) and Sox 8 (Maka et al. 2005), have been identified in HD patients. The interrelationships between the factors is complex and involve processes within the intestinal microenvironment and the viability of neural crest cells (Kapur 1999b).

Most cases of HD are diagnosed in the neonatal period. Ghosh and Griffiths (1998) even suggest that rectal biopsy is not warranted in constipated infants over 1 month of age, although this has not been our experience. Hackam et al. (2004) suggest that later presentation is a reflection of a milder form of the disease. There is a recognized association between HD and congenital abnormalities, particularly Down syndrome and neurocristopathies such as Waardenburg syndrome type 4, neuroblastoma, and multiple endocrine neoplasia type 2. Other abnormalities are less common (Sarioglu et al. 1997).

Failure of completion of migration of neuroblasts from the cephalic neural crest down the alimentary tract is probably the embryological basis of this disorder. Failure of enteric migration has been attributed to a hostile microenvironment caused by an abnormal extracellular matrix (Fujimoto et al. 1989; Parikh et al. 1992). An adequate population of neural crest cells is also necessary (Kapur 1999b). The earlier neuronal migration ceases, the longer the aganglionic segment will be. Despite the absence of intrinsic ganglion cells, extrinsic nerve processes still enter the bowel wall where they proliferate and stimulate contraction, resulting in functional obstruction of the aganglionic segment (Sullivan 1996). Classification depends on the length of the aganglionic segment: short-segment disease involves the rectum and sigmoid colon; in long-segment disease, aganglionosis extends more proximally than the sigmoid colon. Rarely, the entire intestinal tract is aganglionic. The aganglionic segment is confined to the rectosigmoid region in about 70% of cases. There is poor correlation between the degree of obstruction and the length of the segment affected (Garrett et al. 1969).

The use of rectal submucosal suction biopsies to replace full-thickness biopsies marked an important advance in the management of HD. Suction biopsy does not require general anesthesia, is associated with a lower incidence of complications, and, being superficial, does not interfere with subsequent pull-through procedures.

The diagnosis of HD presupposes knowledge of normal innervation of the lower rectum and anal canal. Normally there is a 2- to 3-cm-long aganglionic zone immediately above the mucocutaneous junction of the anus (Weinberg 1975). The length of the physiological aganglionic zone varies with the age of the infant. Biopsies taken from this area may be wrongly diagnosed as HD. Absence of ganglion cells above this level, often accompanied by thickened nerve fascicles, is required for diagnosis (Fig. 18.21), so that an adequate biopsy is important. The thickened nerve fascicles repre-

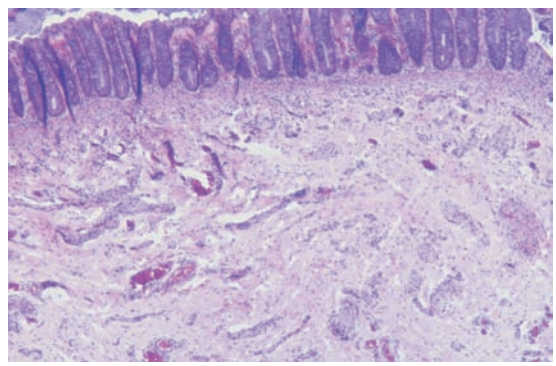


FIGURE 18.21. Hirschsprung's disease. Hematoxylin and eosin (H&E)-stained rectal biopsy shows thickened nerves within the submucosa.

sent sacral parasympathetic outflow and are consequently observed only up to the level of the splenic flexure. When aganglionosis is present and more proximal, the plane of the plexus is empty. A transitional zone of variable length, comprising hypoganglionosis and thickened nerve fascicles, often intervenes between distal aganglionic segment and normally innervated bowel. Another diagnostic pitfall is the presence of immature ganglion cells. They have sparse cytoplasm, lack prominent nucleoli, form rosette-like structures, and may be overlooked. These appearances may persist up to the end of the first year of life (Qualman and Murray 1994).

The acetylcholinesterase reaction has substantially facilitated evaluation of suction biopsies; the results are usually easy to interpret. Cholinergic fibers, particularly in the muscularis mucosae and the submucosa, are increased in number, thickness, and intensity of staining (Fig. 18.22). This appearance extends into the physiological aganglionic zone. In addition, ganglion cells, which are normally easily demonstrated by this method, are absent. Many acetylcholinesterase-positive fibers are present in the lamina propria, but may be absent in the early neonatal period (Huntley et al. 1982; Goto et al. 1984). This is an effective screening method for HD, but false-negative and equivocal results were encountered in 6% of biopsies by Barr et al. (1985). False-negative results are more likely when the biopsy does not include muscularis mucosae or is taken too high in very-short-segment disease. False-negative reaction is rarely encountered in HD and when present is often associated with long-segment/total colonic involvement. Appendiceal perforations is a rare neonatal complication of HD (Martin and Perrin 1967).

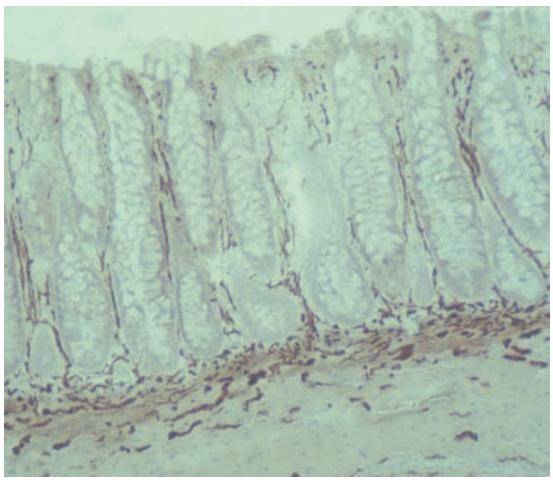


Figure 18.22. Hirschsprung's disease. Frozen section of a rectal biopsy. The acetylcholinesterase method defines thick, short, angulated nerves within the muscularis mucosae and thin, parallel nerve filaments running between glands within the lamina propria of the mucosa.

Controversy surrounds the use of the term *ultra-short-segment HD*. Some view it as a functional defect of the internal anal sphincter; the diagnosis is not made on rectal biopsy as there is no aganglionic segment (Neilson and Yazbeck 1990). Others have defined it as aganglionosis affecting the most distal part of the rectum (Ballard 1996).

Hirschsprung's Enterocolitis

Enterocolitis is the most serious complication of HD, and is found as frequently as 34% (Elhalaby et al. 1995). The condition carries a significant morbidity, but mortality has declined considerably in recent years. Clinical features include abdominal distention, explosive diarrhea, vomiting, fever, lethargy, rectal bleeding, and colonic perforation (Elhalaby et al. 1995). Toxic shock, septicemia, perforation, and peritonitis are common sequelae.

Enterocolitis may be the first presentation of underlying HD. It may precede or follow operative intervention and is more common in long-segment HD (Elhalaby et al. 1995), among females, and in patients with Down syndrome (Carneiro et al. 1992). Recurrent episodes occur.

Three histological patterns have been reported. The usual pattern is extensive necrosis and ulceration of the colonic mucosa (Fig. 18.23) of normally innervated bowel with marked dilatation and variable involvement of the terminal small bowel. The mucosa of the aganglionic segment is little affected. Early histological changes (Teitelbaum et al. 1989) are crypt dilatation, mucin retention, cryptitis, and crypt abscess formation. Pseudomembranous colitis, another microscopic pattern, occurs without preceding antibiotic treatment, and carries a higher mortality (Brearly et al. 1987). Necrotizing (ischemic) enterocolitis, indistinguishable from the usual form of neonatal necrotizing enterocolitis apart

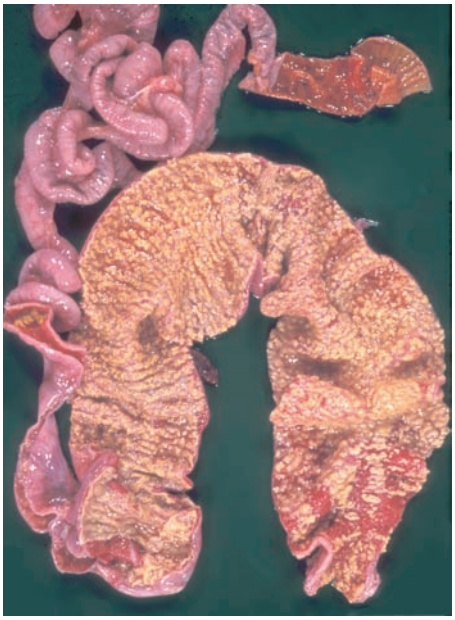


Figure 18.23. Enterocolitis complicating Hirschsprung's disease. The whole (ganglionic) colon is thickened and dilated with extensive mucosal necrosis and ulceration. The distal (aganglionic) segment is spared.

from the low incidence of pneumatosis intestinalis, has also been reported (Teich et al. 1986) It is associated with a more serious outcome.

The pathogenesis of the enterocolitis is poorly understood. Ischemia following mechanical obstruction (Bill and Chapman 1962), a Schwartz-Mann reaction involving bacterial antigens (Berry and Fraser 1968) and *Clostridium difficile* toxin (Thomas et al. 1986) have been suggested; other proposed mechanisms include defective white cell function, deficient transfer of secretory immunoglobulin A (IgA) across the gastrointestinal mucosa and hypersensitivity to absorbed bacterial toxins, increased susceptibility to bacterial invasion due to deficient secretion of acidic sulfomucins and predisposition to the adherence of enteropathogenic organisms and release of toxins (Qualman et al. 1997).

Intestinal Neuronal Dysplasia

Intestinal neuronal dysplasia is a poorly defined condition with aberrant innervation that clinically may simulate HD (Meier-Ruge 1974). Hyperplasia of the intramural plexuses and giant ganglia are described. Isolated ganglion cells are present in the lamina propria and within the muscularis mucosae. Rarely, the whole colon is involved (Dickson and Variend 1983). It may occur in isolation, occasionally coexist with HD (Scharli and Meier-Ruge 1981), and rarely is associated with neurofibromatosis or multiple endocrine neoplasia type IIb or type III (Qualman and Murray 1994). The status of intestinal neuronal dysplasia as a distinct clinicopathological entity is questioned as the histological features originally described have been found to be non-specific, perhaps secondary or part of the normal histologic spectrum (Kapur 2001, 2003).

Megacystis–Microcolon–Intestinal Hypoperistalsis Syndrome

Megacystis–microcolon–intestinal hypoperistalsis syndrome (MMIHS) is a rare cause of intestinal obstruction in the newborn (Wiswell et al. 1979). The outcome is almost always fatal. The abdominal distention is caused by a non-obstructed distended bladder. A malrotated microcolon is associated with normal or increased numbers of ganglion cells. The condition is probably a manifestation of hollow visceral myopathy (Puri et al. 1983) and is characterized histologically by the presence of vacuolar degeneration of muscle fibers and interposed connective tissue (Srikanth et al. 1993). On the basis of more detailed investigation on a full-thickness biopsy of the intestinal wall, the smooth muscle changes of the condition have been shown to be more complex, and several categories have been identified (Smith and Milla 1997).

Anorectal Malformations

Anorectal malformations are among the most common congenital abnormalities of the gut, the incidence being approximately 1 in 5000 live births (Partridge and Gough 1961). Males are more frequently affected than females. Associated chromosomal abnormalities are rare (Robertson et al. 1994). No general agreement exists as to the most satisfactory classification. A core classification agreed to at an international workshop meeting in Wisconsin in 1984 is presented in Figure 18.24 (Stephens and Durham Smith 1986).

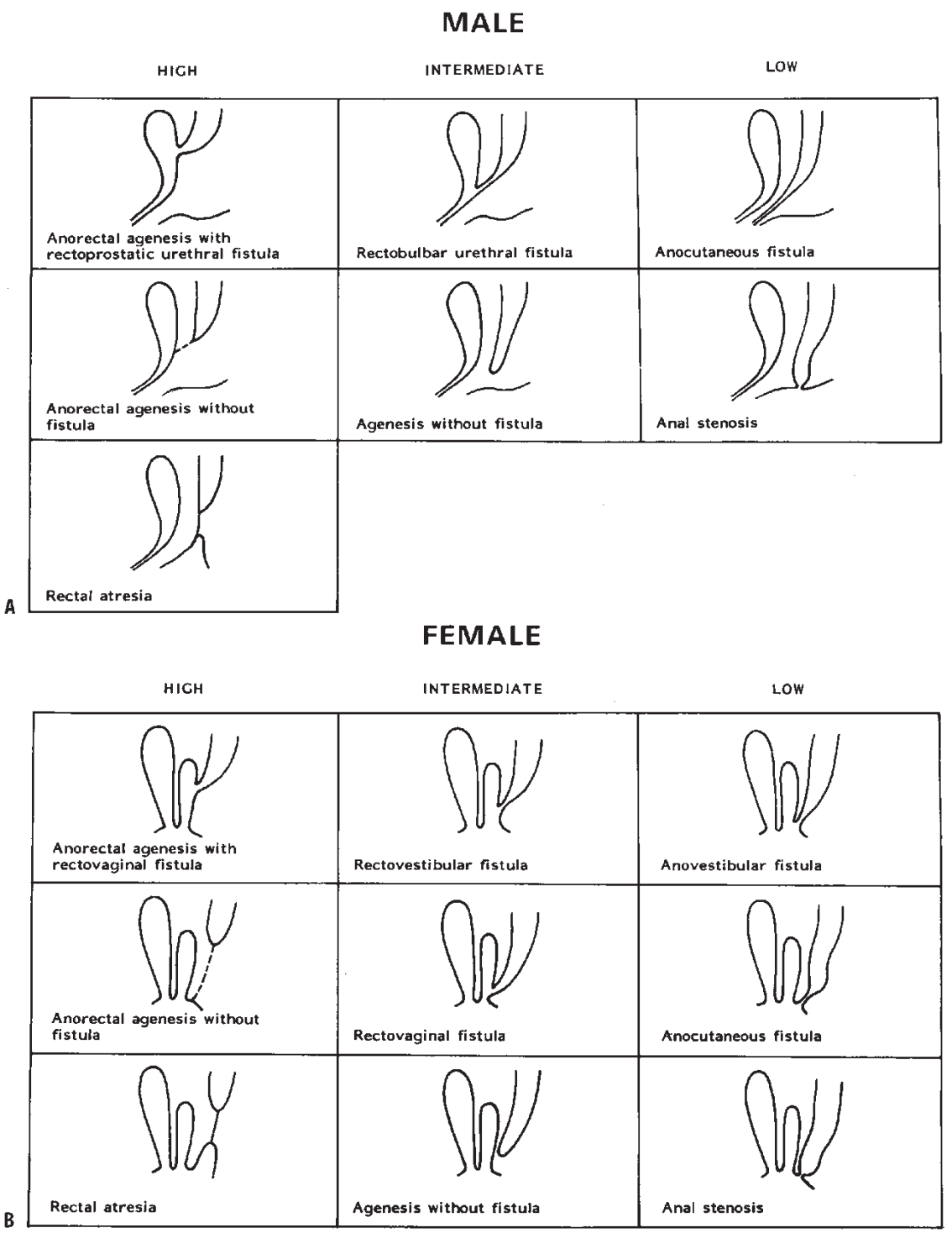


Figure 18.24. Classification of anal atresia and related anomalies. (A) In males. (B) In females. (From Stephens and Durham Smith 1986.)

Abnormalities may be categorized generally as high, intermediate, or low, relative to the level of the puborectalis sling of the levator ani. All types may be associated with fistulas, which may open into the bowel, perineum, vagina, urethra, or, rarely, the bladder, depending on the sex and the level of the lesion.

Associated congenital abnormalities are more common with high malformations, frequently involving the genitourinary system, heart, and skeleton (Gough 1961; Partridge and Gough 1961). Approximately one- third of rectal cases have a serious urological problem (Durham Smith 1968). Abnormalities of the lumbosacral spine are found in over 50% of the "high anomaly" group and include sacral agenesis or hemisacral anomalies. The inclusion of anorectal malformation in the VACTERL association has been alluded to earlier. An imperforate anus occurs regularly in sirenomelia (Stocker and Heifetz 1987).

It has been suggested (van der Putte 1986) that a defect in the dorsal cloacal membrane interferes with its migration toward the tail groove, and that different types of anomalies are produced, depending on the form and size of the defect. Thus, superficial defects cause low malformations such as stenotic anus and ectopic anus, while deeper defects cause anal and anorectal agenesis, with and without fistulas. Administration of a vitamin A analogue to murine embryos resulted in defective cell proliferation in the cloacal membrane and rectourethral or rectocloacal fistulas. Other anomalies such as high anorectal and rectal atresias cannot be readily explained on this basis and, in common with more proximal atresias, is probably due to an ischemic insult in utero.

Agenesis of the whole cloacal membrane results in absence of anorectal and urethral orifices and is the basis of the cloacal dysgenesis sequence; it is usually found in females (McFadden and Pantzar 1992). An association between cloacal abnormalities and incomplete monozygotic twinning has led to the suggestion that partial or complete duplication of the organizing center within the embryonic disk might increase the risk of mesodermal insufficiency, leading to failure of cloacal membrane development and extrophy or other local abnormality (Siebert et al. 2005).

Miscellaneous Conditions

The intestinal tract is the seat of a number of congenital tumors, which include teratomas, hemangiomas, and fibromatoses (Coffin and Pappin 1997; see Chapter 15). The storage product of some lysosomal disorders may be demonstrated in the enteric neurons, and while this does not usually result in symptomatic disease, it does provide suitable biopsy material for histological diagnosis (Lake 1981).

Abdominal Wall Defects and Hernias

Exomphalos

Exomphalos (omphalocele), the most common anterior abdominal wall defect, is a hernia at the base of the umbilical cord. About 1 in 3500 live births are affected. Its sac is formed from amniotic membrane and peritoneum. Small defects contain only loops of small intestine, while other viscera, particularly the liver, are frequently present within the sac of a large defect (Fig. 18.25).

The embryogenesis is poorly understood, but it is generally accepted that the defect results from

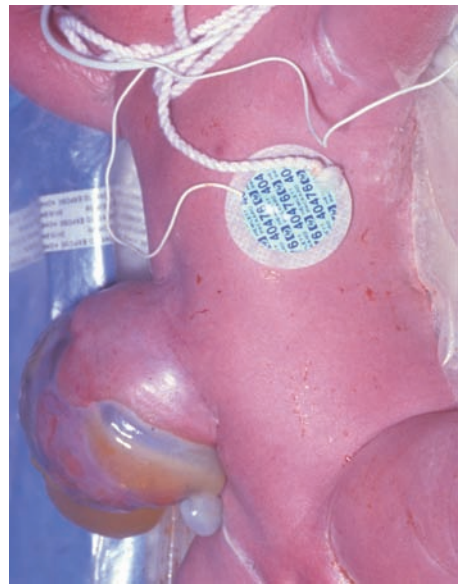


Figure 18.25. Exomphalos major. A large, wide necked sac has the umbilical cord and umbilical vessels running in its wall. (Courtesy of Mr. G. Mackinley, Edinburgh.)

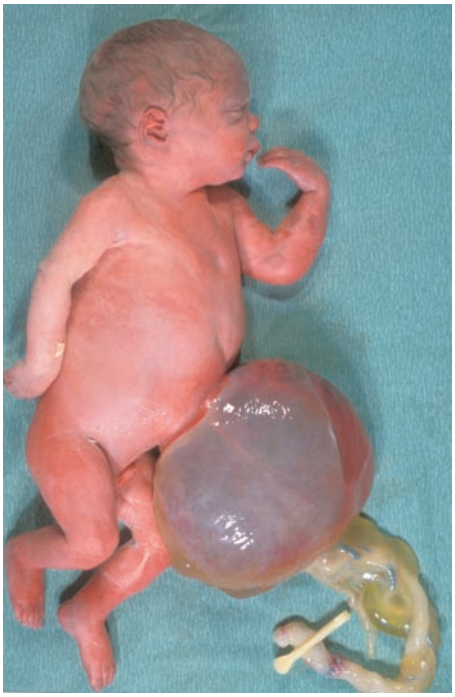


Figure 18.26. Exomphalos in Beckwith-Wiedermann syndrome. The defect in the abdominal wall is small but the sac has stretched and contains much fluid.

failure of the midgut to return from the extraembryonic celom (physiological hernia) to the abdomen. The diameter of the sac is often larger than the abdominal wall defect (Fig. 18.26), it contains no muscle, and is easily stretched. The rectus abdominus muscles are displaced laterally with large defects.

Fetal chest deformity and pulmonary hypoplasia confer poor outcome in babies with large defects (Hershenson et al. 1985). About half of babies with exomphalos have other defects (Boyd et al. 1998). Congenital heart disease has been reported in 24% of cases of exomphalos and is likely to be more complex when the hernia also includes liver tissue (St-Vil et al. 1996).

However, karyotypic abnormality is more common with a small bowel containing hernias (Nyberg et al. 1989; Benacerraf et al. 1990; St-Vil et al. 1996). It is possible that exomphalos with and without liver content may represent abnormalities that are pathogenetically diverse. Exomphalos is frequently seen in trisomies 13, 18, and 21 and Beckwith–Wiedemann syndrome (Fig. 18.26). Prenatal ultrasonography can distinguish exomphalos as early as 12 menstrual weeks (Nyberg et al. 1989).

The sac may rupture before, during, or after birth (Aitken 1963). When antenatal rupture occurs, the exposed loops of bowel are often edematous, friable, and matted together. It is important to distinguish between antenatal rupture of an exomphalos and gastroschisis, as malformations in other systems (Greenwood et al. 1974) and chromosome anomalies (Mann et al. 1984; Nicolaides et al. 1986) that frequently accompany exomphalos are rarely seen with gastroschisis. This will influence both choice of treatment and survival.

Gastroschisis

Gastroschisis is much less common than exomphalos. Preterm delivery is common and chromosomes are usually normal. Antenatal ultrasound examination now provides an effective method of prenatal diagnosis (Nakayama et al. 1984). Theories of pathogenesis include rupture of a small exomphalos (Nakayama et al. 1984), premature involution of the right umbilical vein (deVries 1980), and interruption of the omphalomesenteric artery during early embryonic development (Hoyme et al. 1981). The incidence of gastroschisis has increased in recent years. An association with cigarette smoking in young mothers is suggested (Suita et al. 2000).

There is a full-thickness defect of the anterior abdominal wall usually just to the right of the midline (Shaw 1975; King et al. 1980), generally measuring less than 5 cm in diameter. No sac or sac remnant is present at its margin. A narrow strip of skin often separates the defect from the umbilical cord. Intestinal loops prolapse through the defect and float freely in the amniotic cavity. At birth, eviscerated bowel is encased in a peel of fibrin. The liver is usually intracorporeal. Because of the small defect the exteriorized bowel is prone to vascular obstruction and infarction (Fig. 18.27).

Associated defects are local, not common (Boyd et al. 1998), and are probably ischemic in origin and secondary to the abdominal wall defect. They are confined to the intestine and include jejunal or ileal atresia and stenosis (Amoury et al. 1977). Bowel length is often reduced, and intestinal rota-

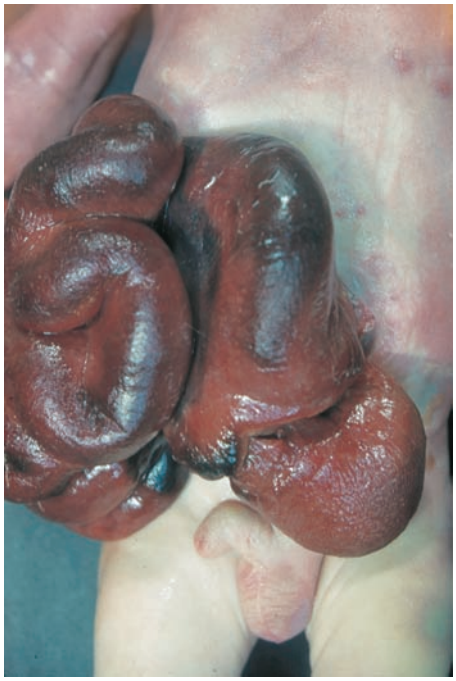


Figure 18.27. Gastroschisis. The exteriorized bowel is dilated and infarcted.

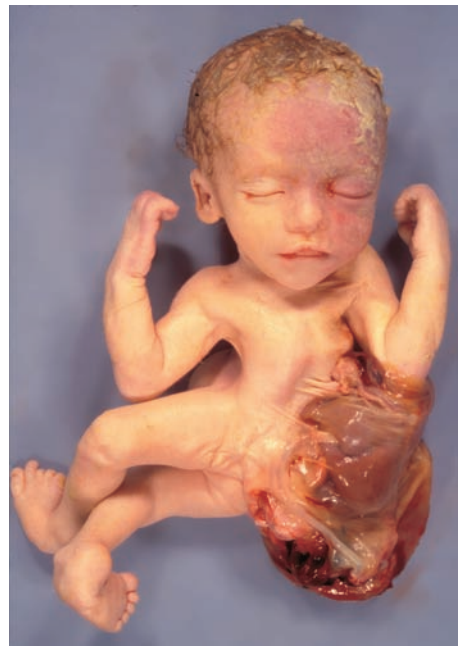


Figure 18.28. Omphalocele-bladder extrophy-imperforate anus-spinal defects (OEIS complex) anomalies. The fetal abdominal wall is closely applied to the chorionic plate. The umbilical cord is short and runs in the wall of the sac.

tion is incomplete. Survivors are susceptible to intestinal motility and malabsorptive problems.

Other Abdominal Wall Defects

Large abdominal wall defects may occur either above or below the umbilicus as manifestations of early amnion rupture (Higginbottom et al. 1979). In the so-called body-stalk anomaly, the fetus is closely applied to the chorionic plate (Fig. 18.28). Umbilical vessels are short and abdominal viscera are exteriorized. The pelvis and lower limbs are usually hypoplastic, and major spinal deformity is usual. The condition is incompatible with postnatal life. Abnormalities referred to as "limb-body wall deficiency" (Miller et al. 1981) and "limb-body wall malformation complex" (Hartwig et al. 1989) are probably related disorders. A nonrandom association of anomalies, omphalocele-bladder extrophy-imperforate anus-spinal defects (OEIS complex), is similar (Kallen et al. 2000). It has been observed in siblings (Smith et al. 1992). Color Doppler sonography can help to delineate the course of the umbilical arteries and may aid prenatal diagnosis (Wu et al. 2004)

In vesicointestinal fissure (lower midline defect) exstrophic hemibladders, each with a ureteric orifice, lie on either side of a central zone of intestinal mucosa with upper and lower orifices (Fig. 18.29) communicating with proximal and distal intestine (Jones 1963; Johnston and Penn 1966).

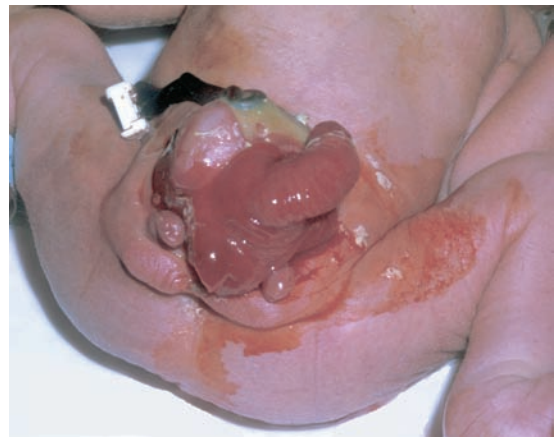


Figure 18.29. Vesicointestinal fissure. The intestines communicate with the upper part of the defect and the ureters with the lower part. The scrotum is bifid and the anus imperforate.

The length of the small intestine is usually reduced and an exomphalos is usually present above the defect. The anus is imperforate and abnormalities of the urogenital system are common. The condition has been referred to erroneously as cloacal extrophy.

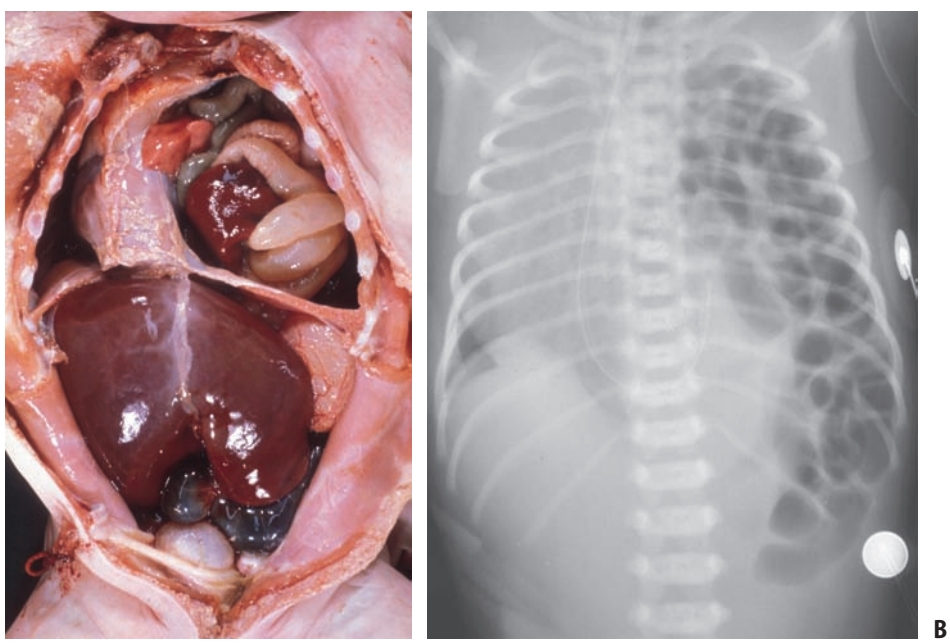
An upper midline defect is frequently accompanied by defects in the lower sternum, pericardium, and anterocentral part of the diaphragm together with cardiac displacement (Cantrell et al. 1958; Toyama 1972). A large exomphalos may be present.

It is important to recognize this group of disruption deformities, which may be accompanied by exomphalos as, despite their major proportion, recurrence is unlikely.

Diaphragmatic Hernia

A defect in the posterolateral part of the diaphragm (foramen of Bochdalek) is due to failure of the pleuroperitoneal canal to close, resulting in persistent communication between the pleural and peritoneal cavities (Fig. 18.30A) (Avery et al. 1981). The majority of diaphragmatic hernias are left sided (about 80%), perhaps related to earlier closure of the right pleuroperitoneal canal. Occasionally, the defect is bilateral. Abdominal viscera become displaced upward into the pleural cavity when the physiological hernia reduces. The pleural cavity on the affected side contains small intestine, proximal colon, stomach, spleen, and, when the defect is large, the left lobe of the liver. The heart is displaced to the opposite side. Pulmonary hypoplasia is usual and, while more severe on the side of the defect, the contralateral lung is usually affected; survival is dependent on the degree of pulmonary hypoplasia. Underdevelopment of the left side of the heart due to intrauterine compression may also be an important factor in mortality (Siebert and Beckwith et al. 1984). The abdomen is scaphoid, with appreciable displacement of viscera into the chest.

Presentation is related to the size of the defect and degree of pulmonary hypoplasia. Large left-sided defects are often recognized at birth because of difficulty in establishing respiration, and others become apparent during the first 24 hours of life



A B

Figure 18.30. Left-sided diaphragmatic hernia (Bochdalek). (A) The small intestine is present in the left hemithorax, the mediastinum pushed to the right, and the hypoplastic left lung is present at the left apex. (B) A radiograph shows mediastinal displacement and air-filled bowel lops in the thorax.

as air swallowing increases the volume of the displaced viscera and produces respiratory embarrassment (Fig. 18.30B). Right-sided defects are often small and, partly protected by the liver, may be asymptomatic; however, when the liver is displaced upward through a large defect, obstruction to the fetoplacental circulation may result in hydrops (see Chapter 14).

Associated congenital anomalies (unrelated to the defect itself) occur in some 73% of cases (Thurlbeck 1992). These malformations predominantly involve the cardiovascular, genitourinary, and central nervous systems.

Survival among those infants effectively transferred to surgical units has improved, reaching 83% in one study (Javid et al. 2004), with 90% survival in busy centers. However, pulmonary development remains the critical factor.

Parasternal diaphragmatic hernias (Morgagni hernias) are far less common and are rarely symptomatic in the newborn (Pokorny et al. 1984), but massive pericardial effusion can be problematic (Fig. 18.31).

Eventration of the diaphragm, when the upwardly displaced abdominal viscera are covered by a sac consisting of abnormally thinned central part of the diaphragm, is much less common.

Exocrine Pancreas

Development

The pancreas develops from the dorsal and ventral outpouchings of the most caudal part of the embryological foregut. Unequal growth of the circumference of the duodenum and rotation carry the dorsal primordium to the left. The ventral primordium initially comes to lie on the right and, with further differential growth of the duodenal wall, joins the dorsal pancreas. Fusion of the two segments occurs in the second intrauterine month. The bulk of the pancreas is formed by the dorsal segment, while the ventral segment forms the uncinate process and the inferior part of the pancreatic head.

Developmental Anomalies

Pancreas Divisum

Pancreas divisum results from failure of fusion of the ventral and dorsal primordia, so that the bulk of the gland (dorsal segment) drains separately through the duct of Santorini, while the isolated ventral pancreas drains through the ampulla of Vater. An association between this malformation

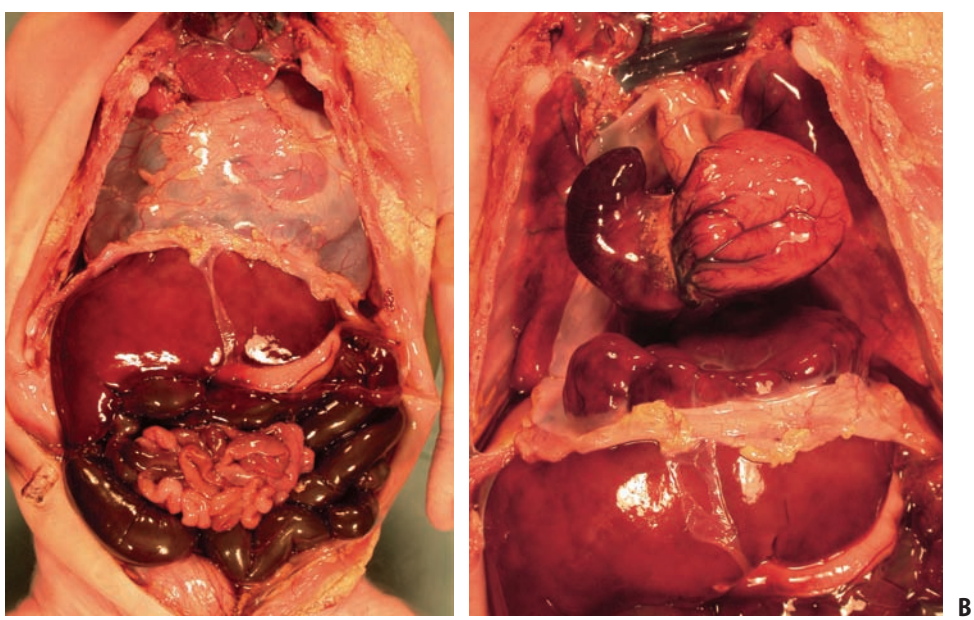


Figure 18.31. Morgagni hernia. (A) There is a large pericardial effusion, a transudate across the liver capsule. (B) Pericardial sac opened. The liver protrudes through the central diaphragmatic defect into the sac.

and pancreatitis in later life has been reported (Cotton 1980). Rarely, the dorsal or the ventral primordium fails to develop.

Annular Pancreas

Annular pancreas is an uncommon abnormality; a ring of pancreatic tissue partially or completely encircles the second part of the duodenum. The lesion is encountered at all ages and may be found incidentally at necropsy (Morrell and Keynes 1981). It frequently accompanies duodenal stenosis or atresia and may rarely cause extrinsic duodenal obstruction. Associated defects are those encountered in duodenal atresia (Jackson 1963).

Ectopic Pancreatic Tissue

Ectopic pancreatic tissue consists of nodules of pancreatic acini within the wall of stomach or small intestine and are a common histological finding in the wall of a Meckel's diverticulum. They are often submucosal in site, but subserosal nodules may be encountered in pylorus and duodenum in trisomies 13 and 18. They are occasionally reported deep to the umbilicus and may cause local discharge (Tan et al. 2000). Focal dysplasia comprising ill-defined areas of immature stroma containing sparse acini and distorted ducts are described in trisomy 18 (Rohde et al. 1964). Inclusions of splenic tissue in the tail of the pancreas are not uncommon. However, they are unduly frequent, in association with microcysts and ducts lined by goblet cells, in trisomy 13 (Hashida et al. 1983).

Other Anomalies

Vestigial pancreas (Elliott and Knight 1974) and congenital absence of the pancreas (Lemons et al. 1979) have also been reported. In the latter condition, intrauterine growth restriction is usually due to the absence or deficiency of insulin, which is an important fetal growth factor.

Mucoviscidosis (Fibrocystic Disease of Pancreas)

The most important disease of the neonatal pancreas is cystic fibrosis. The disease affects exocrine glands throughout the body. Its incidence is around 1 in 2000 births in the U.K. and its inheritance is autosomal recessive. With the discovery of the gene for cystic fibrosis transmembrane conductance regulator *(CFTR),* genotype analysis may become useful technique in diagnosis (Wallis 1997). Clinical manifestations are influenced by genotype, and family studies demonstrate marked concordance of presenting symptoms (Picard et al. 2004).

The gross appearance of the pancreas in the neonatal period is usually unremarkable and histologically the parenchyma is often normal (Oppenheimer and Esterly 1975). These authors reported normal histology in 25% of cases aged up to 6 weeks. The other cases show early changes: inspissated eosinophilic material within acini and ducts associated with epithelial flattening (Fig. 18.32). Fibrosis is not usually a prominent feature at this age. This appearance may not be specific for mucoviscidosis, particularly in preterm neonates (King et al. 1986).

The pancreas is commonly involved in generalized cytomegalovirus infection, and the typical inclusion-bearing cells may be seen in both acinar and islet cells. In erythroblastosis the pancreas is often the seat of marked erythropoiesis. The organ

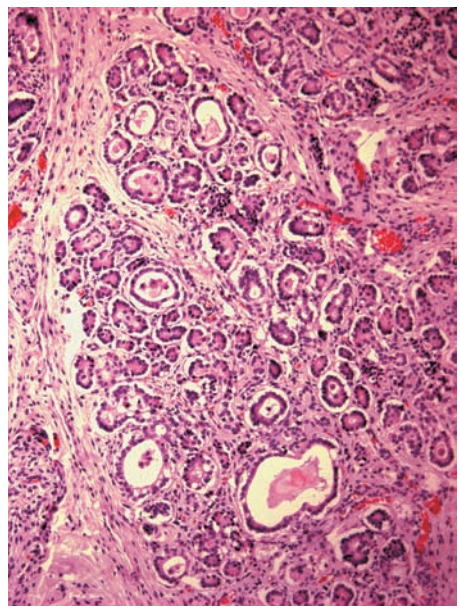


Figure 18.32. Pancreas from a neonate with cystic fibrosis. Small ducts are dilated and filled with secretion, and inspissated secretion is present in acini. There is minimal fibrosis.

is usually affected in congenital leukemia. There is heavy iron deposition in the exocrine glands in neonatal hemochromatosis, an idiopathic neonatal iron-storage disorder (Blisard and Barton 1986; see Chapter 19). Congenital syphilis usually causes pancreatitis, which is characterized by ductular proliferation, acinar loss, and exuberant interstitial fibrosis, and interstitial pancreatitis may form part of the expanded rubella syndrome (Jaffe 1991).

Pancreatic Cysts

Cystic dysplasia of the pancreas has been reported in association with Beckwith–Wiedemann syndrome, Ivemark's syndrome (renal–hepatic–pancreatic dysplasia), short-rib- polydactyly syndrome, and camptomelic dysplasia (Dimmick and Hardwick 1992; Balci et al. 1999, 2000). Pancreatic cysts occur in the Meckel–Gruber syndrome.

Isolated congenital cysts of the pancreas have been described, and microcysts of the pancreas may be seen in association with infantile polycystic disease of the kidneys (Cruickshank 1986).

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19
Liver and Gallbladder

Rachel M. Brown

Normal Development of the Liver

Biliary structures of all calibers, and hepatocytes, are derived from endoderm. A hollow midline outgrowth from the ventral aspect of the future duodenum, known as the liver bud or hepatic diverticulum, develops around the third week of embryonic life. The liver bud grows into the septum transversum and the cardiac mesoderm. These structures contribute connective tissues to the developing liver, and appropriate gene expression is required both in the endoderm and mesoderm for normal development. This process is termed mesoderm inductive signaling (Crosby et al. 2002; Costa et al. 2003). In this environment cells from the bud form thick plates of hepatoblasts surrounding sinusoids fed from vitelline vessels derived from the wall of the yolk sac. Sheets of liver cells are initially many layers thick; by 5 months after birth plates are two cells thick; the adult pattern of plates one cell thick will not be seen until at least 5 years of age (MacSween et al. 2002).

Vasculature

The liver grows under the influence of its blood supply. Initially blood is provided by the symmetrical vitelline veins, which ultimately join to form the portal vein. Later blood is supplied by left and right umbilical veins rich in oxygen and nutrients from the placenta. The right umbilical vein then withers away, leaving the left umbilical vein as principal supplier. Blood in the left umbilical vein takes one of three routes: supplying sinusoids in the left side of liver, sinusoids in the right half of the liver via a connection with the left branch of the portal vein, or to the inferior vena cava via the ductus venosus. Ultrasound studies near term reveal that the left lobe receives almost exclusively nutrient rich umbilical vein blood while the right lobe gets only 50% of its supply from the umbilical vein, the remaining 50% coming from the nutrient-poor portal vein (Haugen et al. 2004). The left lobe is therefore significantly better perfused in utero and as such is better able to withstand hypoxic insults. At birth the left umbilical vein becomes the ligamentum teres and the ductus venosus the ligamentum venosum. Hepatic artery branches appear in developing portal tracts first near the hilum then toward the periphery; this spatial and temporal sequence mirrors that seen in developing bile ducts. The artery appears before the definitive bile duct and may be formed at least in part from portal constituents, specifically myofibroblasts, rather than growing into the portal tracts from the hilum (Libbrecht et al. 2002).

Biliary System

Canaliculi

Canaliculi appear as intercellular spaces between adjacent hepatocytes. They can be seen before bile production begins at 12 weeks' gestation.

Intrahepatic Ducts

At approximately 8 weeks' gestation hepatoblasts around the margins of the mesenchyme of the portal tracts become smaller and strongly express cytokeratins (CKs) types 8, 18, and 19. This sleeve of cells surrounding the portal vein branch with its associated mesenchyme is the ductal plate. The process begins at the hilum around the largest portal tracts and then proceeds outward. A discontinuous second layer of cells now forms around the first, resulting in a double layer around variable stretches of the portal perimeter. Within this double layer slit-like lumina appear. Hepatoblasts not involved in the evolution of the ductal plate lose the biliary type CK 19 expression and differentiate toward hepatocytes. The CK types 8 and 18 are retained by these cells destined to become mature hepatocytes. The early liver cells are therefore bipotential, capable of differentiating into biliary epithelial cells or mature hepatocytes. Contact with the portal mesenchyme orchestrates the differentiation toward biliary epithelium; the portal myofibroblasts have been implicated specifically (Libbrecht et al. 2002). The unique nature of the portal mesenchyme in inducing this differentiation is evidenced by the fact that ductal plates do not form around the central veins. From 12 weeks' gestation onward the ductal plate is remodeled. This process again begins in the largest portal areas near the hilum and proceeds outward toward the smaller portal tracts. The tubular structures, which have formed in the double-layered ductal plate, become surrounded by portal mesenchyme and separated from the parenchyma. Connections are retained between the newly forming duct in the portal tract and the ductal plate and hence to the canaliculi (canals of Hering). As only a single duct persists, remodeling requires the disappearance of unwanted elements of the ductal plate by apoptosis. At term the remodeling process has only just reached the smallest, peripheral, portal tracts where a ductal plate may therefore persist. Full CK7 expression, characteristic of mature biliary epithelium, is not seen until 1 month postpartum. Failure of the precise scheme of spatial and temporal remodeling gives rise to the ductal plate malformation, which can affect any caliber of portal tract (Desmet 1998). This is discussed below (see Abnormalities of Development of the Liver and Bilary Tract). Periportal cells retain the ability to differentiate toward bile duct epithelium seen as the ductules that appear at the portal tract margins in biliary diseases. Speculation surrounds the origin of the ductules either from metaplasia of mature hepatocytes or biliary epithelial cells, or from stem cells located in the canals of Hering, possibly of bone marrow origin (Thiese et al. 1999; Crosby et al. 2002).

Extrahepatic Bile Ducts and Gallbladder

As the duodenum withdraws from the septum transversum, the stalk of the hepatic bud forms the extrahepatic ducts, continuous at one end (caudal) with the duodenum and at the other (cephalic) with the primitive hepatic sheets. The cephalic end of the bud/stalk forms both hepatic ducts and part of the cystic duct, the caudal end forming the gallbladder, part of the cystic duct, and the common bile duct (MacSween et al. 2002).

Functional Development of the Liver and Physiological Adaptations at Birth

At birth there is a shift from placental to enteral nutrition. This stimulates bile acid secretion and the onset of the enterohepatic circulation. The switch from umbilical venous to portal blood supply means new molecules and bacteria arrive via the portal vein to a liver that still has immature metabolic pathways at term. This is illustrated by the immaturity of bile formation and hence by the physiological jaundice that can be observed. It is a vulnerable time for the liver, particularly in the presence of prematurity, hypoxia, sepsis, drug administration, or total parenteral nutrition (TPN) (Suchy and Narkewicz 2002; Beath 2003; Knisely 2003). Hepatocytes are arranged in plates that are thicker than those seen in adulthood but nevertheless have specialized sinusoidal and canalicular surfaces. The sinusoidal aspect is in contact with blood, predominantly from the portal vein, and the canalicular surface is involved in the secretion of bile constituents into the canaliculus.

Hepatocytes are also functionally defined by their location within the acinus or lobule, those around the central vein having different functions from those around the portal tract. This zonal specialization is also still developing at birth.

Synthetic Function

α-Fetoprotein (AFP), the main serum protein of the fetus, is synthesized by hepatocytes 25 to 30 days after conception. It is also synthesized by the yolk sac and intestinal epithelium. Levels peak at the end of the first trimester but are still elevated at birth. It is therefore normal to see a raised AFP in the neonate, and this should not raise undue concern regarding potential neoplasia (Beath 2003; MacSween et al. 2002). Albumin levels are near those of the adult at birth, but plasma proteins involved in coagulation are still at low levels, increasing the risk of bleeding.

Bile acids are synthesized from 5 to 9 weeks' gestation, and bile secretion begins at 12 weeks, but canalicular transport mechanisms and the distal apparatus where the bile is extensively modified are still under development for 4 weeks after birth (MacSween et al. 2002; Knisely 2003). γ-Glutamyltransferase, located at the canalicular surface of the hepatocytes, shows a normal slight elevation in the serum in the first few months of life.

Metabolic and Detoxifying Function

In utero the placenta carries out many of the functions ultimately carried out by the liver, for example, removal of unconjugated bilirubin. To cope with this change, hepatic enzymes are rapidly induced at birth. Conjugation reactions, important for the processing of lipid molecules as by-products of metabolism and of drugs, as well as in the metabolism of bilirubin, are usually mature by 2 weeks. The cytochrome P-450 group and peroxisomal enzymes also show early functionality. The first feed stimulates insulin production and storage of glycogen. Term newborns have glycogen stores but these are quickly depleted, making them prone to hypoglycemia and therefore requiring frequent feeds. Free fatty acids can be converted to ketones in the face of hypoglycemia, or they can be stored as triglyceride. Lactic acid should be cleared within 6 hours of birth. Amino acids are taken up from the portal blood and either enter the urea cycle or are used in protein assembly. In acute-phase disease, proteins, for example, fibrinogen, can be particularly raised because the immature liver fails to clear them (Beath 2003).

Hemopoiesis

The liver is the main site of hemopoiesis at 12 weeks, but this role is taken over by the bone marrow from 5 months. It is normal to see a little residual hemopoiesis after birth for up to 6 weeks (Beath 2003). Hemopoietic foci are particularly prominent in neonatal hepatitis (see below). Hemosiderin, which accumulates as hemopoiesis decreases, and copper-associated protein (copper is normally excreted in bile) accumulate in periportal hepatocytes and are still present at birth; that is, both are normal constituents of the neonatal liver and are not reliable in disease assessment.

Abnormalities of Development of the Liver and Bilary Tract

Hepatic Anatomy

Agenesis of the liver is rare and incompatible with life. Agenesis of a single lobe is also rare. An absent right lobe has been reported more frequently than absence of the left, and can be associated with other abnormalities (Radin et al. 1987). Abnormalities of position are seen more often. In situs inversus the liver is present on the on left side, which can be associated with the embryonic (or syndromic) form of extrahepatic biliary atresia (EHBA). The liver can be displaced into diaphragmatic hernias or omphaloceles. Accessory lobes are quite frequent arising from the inferior surface of the liver. Riedel's lobe is a tongue-like projection of the right lobe. Ectopic liver tissue can be seen in the suspensory ligaments of the liver and the lung, the wall of the gallbladder, the splenic capsule, the retroperitoneum, and the greater omentum. Heterotopia also occurs. Most cases of adrenal tissue in the liver are instances of hepatoadrenal fusion where the adrenal capsule is

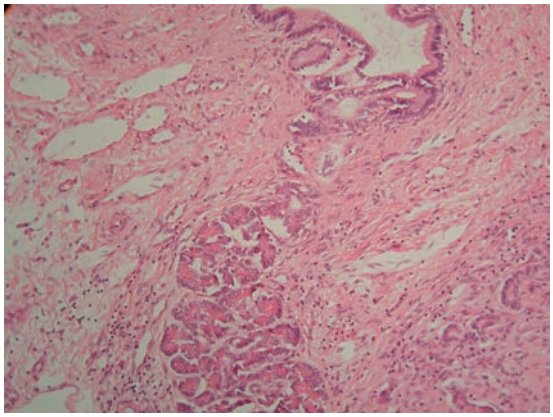

Figure 19.1. Pancreatic heterotopia. Exocrine pancreatic elements are seen adjacent to a bile duct in a large portal tract. [Hematoxylin and eosin (H&E), original magnification ×200.]

lacking. Pancreatic tissue can also fairly frequently be seen within the liver (Ishak and Sharp 2002) (Fig. 19.1).

Vasculature

Defects in the development of the portal vein can lead to its absence, a preduodenal position, cavernous transformation, "cavernoma," duplication, and congenital portosystemic communications. A cavernoma can be accompanied by ductal plate malformation (see below). Portocaval shunts are divided into two types: type I, where portal venous blood is completely diverted from the portal vein to the inferior vena cava (IVC), bypassing the liver entirely, and type II, where only part of the blood flow is diverted to the IVC (Morgan and Superina 1994). Type I shunts are commoner in girls, and they can be associated with other abnormalities, for example, EHBA, and later in life with liver tumors. Type II shunts are less common and present later in life with encephalopathy. Hepatoportal arteriovenous connections can be congenital. Sometimes they occur as part of a syndrome, such as Osler-Weber-Rendu syndrome, or following trauma or biopsy. They can lead to portal hypertension (Stringer and Howard 2004). The effects of abnormal blood flow through the liver of any cause can give rise, in later life, to the histological changes of focal nodular hyperplasia and other lesions in the spectrum of noncirrhotic portal hypertension.

Biliary Abnormalities Including Fibrocystic Diseases

While some cases of paucity of intrahepatic bile ducts and of extrahepatic biliary atresia may be regarded as abnormalities of development, these conditions are usually encountered in the context of a biopsy from a jaundiced neonate and are therefore discussed in the subsequent section.

Structural anomalies are relatively common in terms of the arrangement of the gallbladder, cystic duct, and hepatic ducts, consisting of duplications, accessory ducts, or anomalous insertions. These are felt to be due to abnormal remodeling of the caudal end of the hepatic bud (Knisely 2003).

The most important developmental anomaly to recognize in the biliary tract is the ductal plate malformation (DPM). The process of ductal plate development and remodeling has been described in the first section. The DPM represents a failure in the remodeling process leading to a persistence of ductal plate structures. It is important to bear in mind the following:

1. All levels of the intrahepatic biliary tree from the largest ducts to the smallest are susceptible to DPM.
2. The remodeling process begins near the hilum and progresses toward the periphery (Desmet 1998).

The timing of an insult during fetal life, therefore, determines which parts of the biliary tree are affected and subsequent manifestations. An early and persistent insult to the complex epithelial and mesenchymal interactions involved in remodeling affects ducts of all caliber, whereas an insult later in development only affects smaller ducts. A DPM is often associated with abnormal ramification of the portal vein seen as altered spatial arrangements between portal structures (Desmet 1998).

Histologically the DPM consists of numerous ductules at the portal tract margins. They have slit-like lumina and flattened basophilic-appearing epithelium. They often lie in close proximity to the hepatocytes without intervening stroma. The ductules can anastomose and sometimes contain

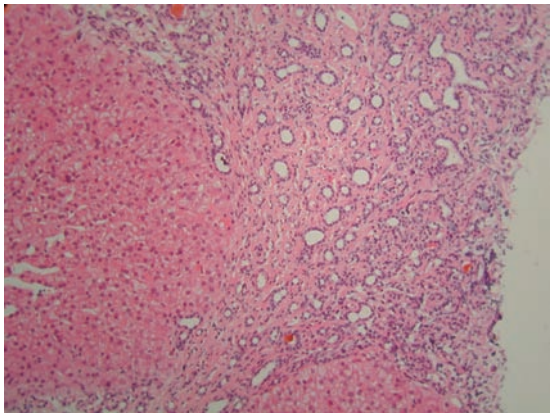

Figure 19.2. Ductal plate malformation. This is a feature of many different syndromes associated with varying degrees of fibrosis. Numerous ductules are seen with basophilic epithelium, some containing secretions. Note the normal parenchyma on the left. (H&E, original magnification ×200.)

inspissated material (Fig. 19.2). A portal vein branch is usually identifiable or several branches may be seen, but an appropriately sized bile duct (of similar caliber to, and adjacent to, the hepatic artery branch) cannot always be seen as, by the nature of the DPM, remodeling that would have led to the formation of a definitive duct has been stalled.

A DPM is often seen in association with variable degrees of duct ectasia and fibrosis referred to as fibrocystic diseases. The cysts, being ectatic ducts, usually communicate with the biliary tree. Morphological features of the different entities overlap, with the DPM as the consistent finding. Fibrocystic diseases occur in isolation or more commonly with lesions in other organs, usually the kidney, as part of a syndrome. As understanding of the genetic basis of these diseases is emerging, classification is moving away from clinical/morphological terms to genetic classifications.

In the neonate conditions associated with the DPM are autosomal recessive polycystic kidney disease (ARPKD), Meckel-Gruber syndrome, and other uncommon lethal anomalies (Dimmick and Jevon 1998).

Syndromes Associated with Ductal Plate Malformation

The gene responsible for ARPKD ease is *PKHD1*, located on chromosome 6. It is a large gene with multiple different disease-causing mutations. The protein product is fibrocystin, the function of which is unknown. Possibilities include a receptor for growth factors, because of the predicted extracellular domain, or a role in cell adhesion (Johnson et al. 2003; Harris and Rossetti 2004). Fibrocystin is expressed in the branching ureteric bud, renal collecting ducts, and bile ducts. In the kidney it is localized to the cilia of renal epithelial cells, suggesting a link between ciliary function and cyst development (Harris and Rossetti 2004). This pattern of expression gives rise to a unifying theory for some of the manifestations of syndromes associated with the DPM. Common pathways may exist for the development of tubular epithelial structures in different organ systems. The diverse proteins identified may interact with each other either to form multiunit complexes or to mediate signal transduction or adhesion events between epithelial cells or between epithelial cells and extracellular matrix. If a common molecular mechanism does exist, there must be variations that determine phenotype and the altered prognosis that is observed within each phenotype (Johnson et al. 2003).

Autosomal recessive polycystic kidney disease encompasses a clinical spectrum of disorders. All include the DPM in the liver and cystic dilatation of the renal collecting ducts. The various types present at different ages (perinatal, neonatal, infantile, and juvenile); the younger the age of presentation, the more severe the renal lesion, and it is the renal lesion that governs outcome. With increasing age the liver lesion is more manifest such that the variant with juvenile onset is regarded as the equivalent of congenital hepatic fibrosis (CHF). Another pattern, which can be seen in the older child, is the combination of CHF and dilatation of the large intrahepatic bile ducts, Carolis syndrome, which is also regarded as part of the ARPKD spectrum (Kniseley 2003). Scarring in both the liver and kidney continues after birth, implying either an ongoing injurious insult or secondary effects, which, in the liver, would include the consequences of biliary obstruction.

Histologically the CHF liver shows a jigsaw pattern of lobulated nodules separated by dense fibrous tissue (Fig. 19.3). The DPM is seen in and at the margins of septa. In the uncomplicated case

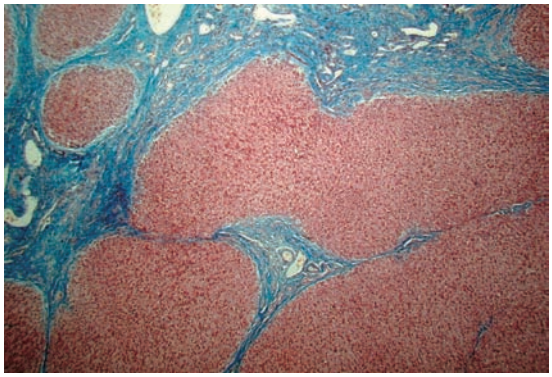

Figure 19.3. Congenital hepatic fibrosis. Jigsaw like nodules are separated by dense fibrous tissue septa that contain the ductal plate malformation. (Masson trichrome, original magnification ×100.)

the parenchyma is strikingly normal with an absence of canalicular cholestasis.

Other syndromes associated with DPM can be divided into those associated with skeletal abnormalities and those with central nervous system (CNS) abnormalities.

Skeletal Malformation Syndromes

Jeune asphyxiating thoracodystrophy (JATD) features a long narrow thorax, metaphyseal defects, and shortened ribs and long bones. Pulmonary hypoplasia may cause early demise. Pancreatic cysts can be seen. Ellis–van Creveld syndrome (EvC) is similar to JATD but with polydactyly, fingernail dysplasia, and cardiac defects as characteristic. The EvC gene has been mapped to chromosome 4p16, and the protein product is of unknown function. No gene is known for JATD or for the variants of short rib polydactyly syndrome (SRPS). Bardet-Biedl syndrome features skeletal abnormalities in terms of polydactyly; multiple genetic loci have been identified (Johnson et al. 2003; Knisely 2003).

Central Nervous System Malformation Syndromes

In Meckel-Gruber syndrome a triad of CNS abnormalities (prosencephalic dysgenesis, occipital encephalocele, and rhombic roof dysgenesis) is classically seen with large multicystic dysplastic kidneys, DPM, and polydactyly. Ductal plate malformation and renal dysplasia are the most consistent abnormalities (Sergi et al. 2000). Three potential loci but no specific genes have been identified. A Dandy-Walker–type malformation can also be seen in other syndromes associated with the hepatic DPM, such as hepatic renal pancreatic dysplasia (Ivemark syndrome), SRPS II, and Joubert syndrome (Johnson et al. 2003; Knisely 2003).

Cysts

At least some simple solitary bile duct cysts may be developmental in origin with failure to establish a connection with the biliary tree, but these lesions often only present in adulthood (Ishak and Sharp 2002).

Other rare congenital cysts of the liver include ciliated hepatic foregut cyst, which presumably arises due to the incorporation of foregut structures, proximal to the hepatic bud, into the septum transversum. Incomplete separation of developing foregut structures may also account for the rare bronchobiliary fistula. Intestinal duplication cysts are also rarely observed (Ishak and Sharp 2002).

The term *choledochal cyst* has been used to describe a heterogeneous group of conditions characterized by dilatations of varying portions of the extra- and intrahepatic biliary tree, likely to represent acquired as well as developmental abnormalities. They are more common in oriental races and also more common in girls (3:1). Classically five types of cyst have been described (Todani et al. 1977). By far the commonest (70%) is type I, a cystic (Ic) or fusiform (If) dilatation of the common bile duct. Type IVa cysts are next in frequency, comprising multiple dilatations of intra- and extrahepatic ducts. Types II (diverticulum), III (choledochocele), IVb (multiple extrahepatic cysts), and V (multiple intrahepatic cysts) are rare. Type V corresponds to Carolis disease. The latter has been regarded as a type of DPM involving large ducts only. Etiology in the other types is disputed as is the utility of the above classification system (Stringer and Howard 2004; Visser et al. 2004). An acquired weakness of the bile duct wall secondary to reflux of pancreatic secretion and dilatation secondary to distal stricture have both been proposed. Reflux of pancreatic secretions is precipitated by the pancreatobiliary malunion, which can accompany types I and IV cysts (Iwai et al. 1992). Presentation can be at any age; neonatal presentation is with an obstructive jaundice, and

early surgery to completely remove the cyst and establish hepaticoenterostomy is indicated. Histologically the cyst has a fibrous wall, and the epithelium is often at least partially denuded. Concurrent liver biopsy may show a biliary obstructive pattern with fibrosis, but long-term outlook with prompt surgery is good (Bancroft et al. 1994). Apart from obstruction, longer term complications include perforation, stone formation, and carcinoma. Complete excision of the entire extrahepatic biliary tree is therefore indicated (Visser et al. 2004).

The Jaundiced Neonate

Jaundice is common in the neonatal period. Hyperbilirubinemia can be unconjugated or conjugated. Unconjugated hyperbilirubinemia, where there is no extra pigment present in the urine, can be physiological, activity of the enzyme uridine diphosphate glucuronyl transferase (UDPGT) being suboptimal at birth. It can also be seen in the settings of breast-feeding or in systemic disorders, for example, sepsis (the commonest cause of nonphysiological jaundice; Beath 2003) and hemolysis. Rarely, Crigler-Najjar syndromes (type 1, lack of UDPGT; type 2, partial defects) and the milder Gilbert syndrome, with abnormalities of expression of UDPGT, are implicated in unconjugated hyperbilirubinemia. Galactosemia and fructosemia can also present with an unconjugated hyperbilirubinemia (Beath 2003). Biopsy is rarely performed in these settings, the pathologist is much more likely to be involved in assessing biopsies from neonates with conjugated hyperbilirubinemia, which is almost invariably pathological. Here the urine is dark and the stools are pale. The commonest etiology is extrahepatic biliary atresia. The commonest metabolic cause and most important differential diagnosis is the liver lesion associated with α_1-antitrypsin deficiency. Other conditions are grouped together under the umbrella term of *neonatal* (or *giant cell*) *hepatitis*. Finally, there is the group of abnormalities characterized by a paucity of intrahepatic bile ducts. A decision tree for the assessment of biopsies is outlined in Table 19.1. Some of these conditions overlap with those that can present as liver failure leading either to transplantation or to death. Liver failure and subsequent examination of the liver either at transplantation or at autopsy are dealt with in the next section.

Extrahepatic Bilary Atresia

There is urgency to establishing of a diagnosis of extrahepatic biliary atresia (EHBA), as early surgical correction is related to outcome. It should therefore be the first question in the mind of the pathologist (Table 19.1). Clinically the child will

Table 19.1. This decision tree indentifies four common histological patterns in biopsies from neonates with conjugated hyperbilirubinemia in the context of confirming or excluding a diagnosis of EHBA. In cases which are not EHBA potential etiologies and investigations are suggested. Further discussion, organized by etiology, follows in the text

Are biopsy features those of biliary atresia?			
Fibrous portal tract expansion Marginal ductules with bile plugging Variable inflammation and parenchymal giant cell transformation Normal serum α_1-antitrypsin	Fibrous portal tract expansion and ductular transformation only mild Variable inflammation and parenchymal giant cell transformation	Florid parenchymal giant cell change, cholestasis and extramedullary hemopoiesis Minimal, if any portal biliary features **Neonatal hepatitis**	Dominant pattern is **paucity of intrahepatic bile ducts** Note: paucity can evolve after birth and may not be evident in neonate Peripheral paucity may be normal in prematurity
YES Proceed to early laparotomy for confirmatory cholangiogram and Kasai procedure	NOT SURE Exclude **α_1-antitrypsin deficiency** (paucity of bile ducts and periportal steatosis are potential clues) Correlate with isotope scan results and clinical history **May need repeat biopsy** Consider infantile presentation of cystic fibrosis	NO Biopsy findings can guide clinical investigations Are any clues present: steatosis, pseudoglandular cholestasis, excessive iron deposition, viral inclusions, EM findings?	NO Investigate for syndromic and nonsyndromic causes

usually have had an ultrasound scan, which may have shown an abnormal or absent gallbladder, and also radioiodine studies to determine if the flow of bile into the duodenum is blocked. False-positive results can be seen with the latter test, however, where the extrahepatic ducts are hypoplastic secondary to reduced bile flow. This can happen in some cases of paucity of intrahepatic ducts and neonatal hepatitis. Therefore, the pathologist should always keep an open mind.

Etiology/Pathogenesis

This condition arises due to obliteration of portions of the extrahepatic biliary tree. The obliteration may involve the right and left hepatic ducts and gallbladder, which is the most common pattern, the proximal hepatic duct alone or the distal common bile duct with proximal hilar cysts. It is increasingly recognized that EHBA is not a single entity but represents a stereotyped end-stage response of the fetal/neonatal biliary system to multiple different etiologies (Perlmutter and Shepherd 2002; Kahn 2004). Clinicopathologically two major types emerge: 10% to 30% have an embryonal or syndromic form, where the biliary atresia is associated with other congenital abnormalities such as polysplenia, situs inversus, and preduodenal portal vein, and the majority have a perinatal nonsyndromic form, where the biliary atresia is an isolated phenomenon. Five categories of postulated etiologies are suggested by Perlmutter and Shepherd (2002):

1. Viruses: Candidate viruses have included rotavirus and reovirus. Reovirus type 3 has been identified with increased frequency in tissue from patients with EHBA or choledochal cyst as compared to patients with other forms of neonatal liver disease (Tyler et al. 1998). Viruses are postulated to induce the expression of neoantigens on the biliary tract epithelium, which are then presented to the immature immune system (Sokol and Mack 2001). A role for cytomegalovirus (CMV) in the etiology of EHBA has not been substantiated (Jevon and Dimmick 1999).
2. Environmental toxins: No consistent candidate has been identified.
3. Defects in morphogenesis: Arising from studies of a mouse model with mutations in the inversin *(INV)* gene displaying lateralization defects and progressive jaundice, this gene has been studied in humans (Schon et al. 2002). Initial studies in children with syndromic EHBA associated with lateralization defects have not, however, shown evidence of INV mutations. Mutations of genes involved in organogenesis and spatial relationships remain a possibility. In some cases of EHBA the liver shows features reminiscent of DPM. This can be conspicuous in end-stage disease seen at the time of transplantation. Some forms of EHBA, especially those with an apparently intrauterine onset, may therefore represent abnormal development of the extrahepatic biliary tree associated with intrahepatic DPM (Desmet 1998).
4. Aberrant inflammatory responses: There is no characteristic pattern of inflammation in EHBA as assessed by morphology or routine immunohistochemistry (Davenport et al. 2001). Altered lymphocyte differentiation has been suggested (Bezerra et al. 2002). A human leukocyte antigen (HLA) association that might account for a genetically determined abnormal immune response to environmental antigens has not been confirmed (Donaldson et al. 2002).
5. Vascular insults: Ultrasonographic and histological abnormalities of the hepatic artery and its branches have led to speculation as to whether the biliary damage is secondary to ischemia (Ho et al. 1993).

As intimated above, different etiological factors may be important in different types of EHBA, and in all cases definitive causes are still elusive. In some children the biliary insult may be ongoing in that explanted livers can show a distinct paucity of intrahepatic bile ducts. Extrahepatic biliary atresia might therefore be regarded as a pan biliary disease (Desmet 1998; Knisely 2002). Some authors would argue, however, that this paucity is secondary to ongoing large duct obstruction (Nietgen et al. 1992). The novel composition of bile arriving at the extrahepatic bile ducts immediately after birth damaging immature cholangiocytes (Knisely 2003) and a failure of canalization of the extrahepatic ducts have also been postulated (Suchy and Narkewicz 2002).

Histological Findings

Appearances of the extrahepatic biliary tree, completely removed during the Kasai procedure can vary. Sometimes a large-caliber duct is still iden-

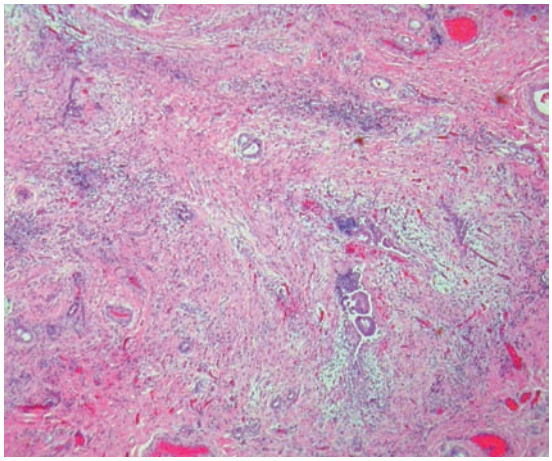

Figure 19.4. Hilar portal plate in extrahepatic biliary atresia. A common appearance is illustrated with scattered ductules in a fibroinflammatory background. (H&E, original magnification ×100.)

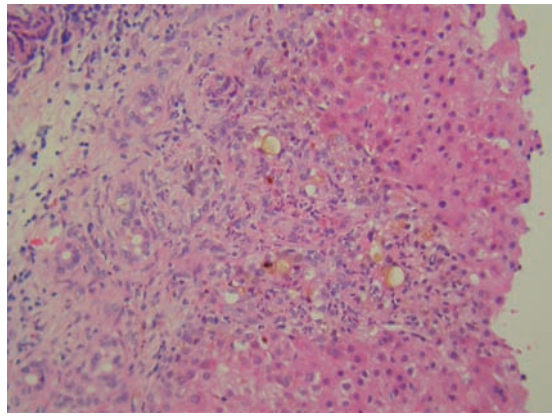

Figure 19.6. Ductular bile plugs in extrahepatic biliary atresia. These are a characteristic finding but are also seen in other conditions, e.g., α_1-antitrypsin deficiency and total parenteral nutrition (TPN). (H&E, original magnification ×200.)

tifiable with an inflamed wall. A completely obliterated fibrous cord is occasionally seen or multiple small ductules are present in a fibroinflammatory background (Fig. 19.4). Assessment of core liver biopsies should be concentrated on the portal tracts where there is fibrous expansion accompanied by ductules at the portal tract margins. These show variable plugging with bile (Figs. 19.5 and 19.6). Usually an appropriately sized bile duct, that is, a bile duct of similar caliber, in close proximity to the hepatic artery can be identified, but sometimes assessment is obscured by the abundance of ductules. Portal inflammation is variable. Marked canalicular cholestasis is seen in the parenchyma often accompanied by parenchymal giant cell change and extramedullary hemopoiesis. These features should not detract from the diagnosis. Periportal iron deposition and copper-associated protein may be identifiable but are of no diagnostic value as they are physiological findings in this age group. It should be stressed that the features described as typical of biliary atresia are simply the features of large duct obstruction, and they would be identical in a choledochal cyst or the inspissated bile syndrome (seen in hemolysis or septicemia), for example. The other point to stress is that the features described can be exactly mimicked in α_1-antitrypsin deficiency. This disorder, therefore, always should be excluded by serum levels and, ideally, protease inhibitor phenotype before proceeding to laparotomy. There are occasions where an early biopsy can be equivocal or misleading, and repeat biopsy is necessary (Azar et al. 2002).

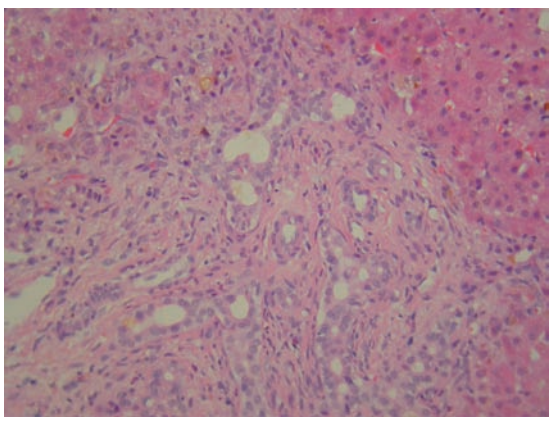

Figure 19.5. Portal tract in extra hepatic biliary atresia. Ductular transformation is seen in an expanded portal tract. (H&E, original magnification ×200.)

Treatment/Prognosis

Once the diagnosis of EHBA is strongly suspected, laparotomy is performed and confirmatory cholangiogram is undertaken. If the cholangiogram is positive, Kasai hepatoportoenterostomy is performed. This involves the complete removal of the extrahepatic biliary tree and anastomosis of the end of a roux-en-Y loop to the porta hepatis. This

can restore bile flow and, when successful, achieve a 10-year survival with the native liver of over 90% (McKiernan et al. 2000). These children can avoid chronic liver disease and achieve a good quality of life (Hadzic et al. 2003). From the Kasai, the pathologist receives the hilar plate, as described above, and often a wedge of liver for staging of fibrosis. Possible features relating to outcome have been investigated, including fibrotic stage and the caliber of the largest residual duct at the hilar plate, but the only consistent variable is the age of the patient at the time of the Kasai. Infants older than 100 days may still benefit from the Kasai procedure, however, surviving with their native liver in a third of cases in the medium term (Davenport et al. 1997, 2004). The experience of the surgical center performing the procedure is also an important prognostic factor (McKiernan et al. 2000). Overall, 50% to 60% of children will go on to require transplantation, EHBA being the most frequent indication for a transplant in childhood (Davenport et al. 1997). Examination of the explanted liver can reveal a typical biliary cirrhosis or show in addition a paucity of intrahepatic bile ducts or features resembling ductal plate malformation.

α_1-Antitrypsin Deficiency

This is the commonest metabolic disorder to present with neonatal cholestasis, and it can have multiple histological patterns. It therefore should always be considered in the differential diagnosis when assessing a biopsy.

Etiology/Pathogenesis

α_1-Antitrypsin (A_1AT) is the main circulating serine protease inhibitor (Pi), its main role being inhibition of neutrophil elastase. Deficiency leads to emphysema and liver disease. There are numerous different alleles encoded on chromosome 14. The most prevalent allele, associated with normal serum levels, is M. Inheritance is in a codominant manner, and the commonest Pi phenotype in disease is PiZZ. PiMZ and SZ, as well as other variants, can also be associated with neonatal liver disease, but this is very rare and in fact only 10% of PiZZ infants develop significant liver dysfunction (Alagille 1984). The Z variants are associated with abnormal protein folding and sequestration in the endoplasmic reticulum. In other variants, for example, S, there is degradation of the protein, and null variants also exist where synthesis is absent. Clinically, levels in serum can be determined rapidly; Pi phenotyping takes a little longer. Serum levels at least should be known before accepting a diagnosis of EHBA on biopsy findings alone.

Histological Findings

In A_1AT deficiency, the biopsy can show one of several patterns. It can be biliary with fibrous portal tract expansion and marginal ductules, some containing bile plugs exactly as seen in EHBA. In this scenario clues to A_1AT deficiency, which are by no means always present, are steatosis, often with a periportal distribution, and paucity of a bile duct proper (Alagille 1984; Filipponi et al. 1994) (Figs. 19.7 and 19.8). It is important to distinguish between an appropriately sized bile duct, as defined above, and ductules at the portal tract margins. Periportal iron deposition is seen in both A_1AT deficiency and EHBA. Bile duct paucity can be present without fibrosis or marginal ductules, and finally the picture can be neonatal hepatitis (see below). Periodic acid-Schiff (DPAS)-diastase positive globules, typical of Z variants in older patients, are not consistently identifiable in periportal hepatocytes in neonates, and it follows that there is little chance of staining

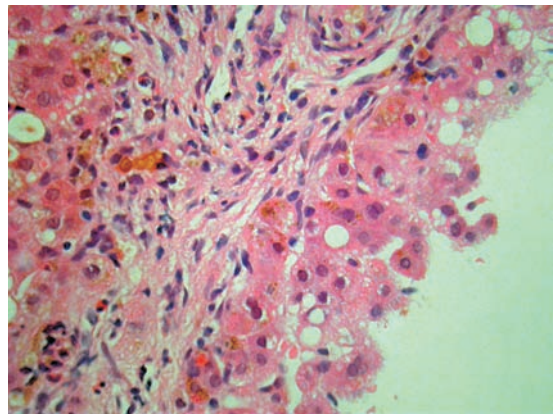

FIGURE 19.7. α_1-Antitrypsin deficiency. Portal tract with biliary features in the form of a ductular bile plug. Note the periportal fat—a potential clue to the diagnosis. (H&E, original magnification ×400.)

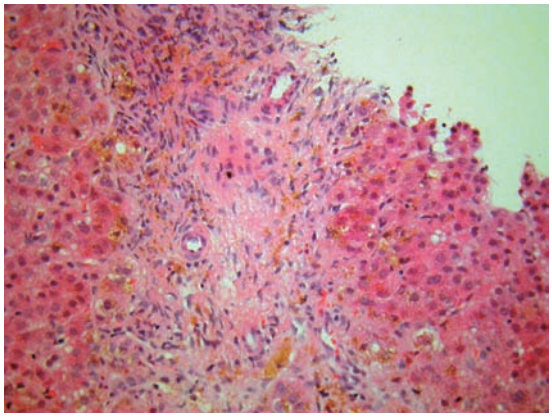

Figure 19.8. α_1-Antitrypsin deficiency. Paucity of bile ducts. Hepatic artery branches are seen, but there is no accompanying bile duct of a similar caliber. Periportal iron pigment is often conspicuous. (H&E, original magnification ×200.)

A_1AT by immunohistochemistry. Interpretation of immunohistochemistry is further complicated by the frequent presence of pigmented periportal iron granules. DPAS-diastase positive material is often present in macrophages (Kupffer cells) whenever there has been parenchymal damage and should not be misinterpreted as true periportal globules (Jevon and Dimmick 1998a) (Fig. 19.9).

Treatment/Prognosis

As noted above, not all neonates with abnormal Pi phenotypes present with jaundice, and among those who do the outcome is unpredictable (Alagille 1984). Biliary histology and bile duct paucity have been claimed to confer a worse prognosis (Alagille 1984; Filipponi et al. 1994; Portmann 1994). Those who do have progressive disease develop cirrhosis, often with biliary features, and are at risk for hepatocellular carcinoma, particularly in males (Portmann 1994). Transplantation has good results (Filipponi et al. 1994).

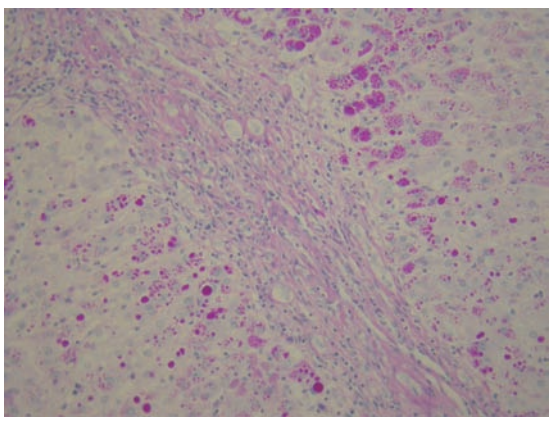

Figure 19.9. α_1-Antitrypsin deficiency. End-stage disease with true DPAS positive globules. These are not seen in the first 3 months of life. (DPAS, original magnification ×200.)

Neonatal Hepatitis

Etiology/Pathogenesis

Neonatal hepatitis does not describe a specific disease; rather, it encompasses many different etiologies, mostly either infectious or metabolic, that induce a similar pattern in a liver biopsy. Often no etiology can be identified. The pathologist should always seek clues in the biopsy that can direct subsequent clinical investigations.

Histological Findings

Architecture is usually preserved unless there is necrosis and collapse. There can be a spectrum of appearances between neonatal hepatitis (NNH) and submassive hepatic necrosis, which is discussed in the next section. Portal tracts contain normal structures and a variable inflammatory infiltrate that can include eosinophils. Parenchymal changes dominate with disarray, canalicular cholestasis, extramedullary hemopoiesis, and giant cell change (Fig. 19.10). These are nonspecific findings; giant cells are thought simply to represent fused pericanalicular, rosette forming, hepatocytes (Koukoulis et al. 1999). As giant cells die off, they leave a cluster of neutrophil polymorphs, which should not be misinterpreted as true microabscesses.

Clues to a Metabolic Etiology

A_1AT deficiency, in which biliary features, bile duct paucity, and periportal steatosis as well as NNH-like changes can be seen, has been discussed above. Steatosis in general should arouse suspicion of a metabolic disorder. Extreme rosetting or pseudoglandular cholestasis accompanied by fatty change can be seen in galactosemia, tyrosinemia, and hereditary fructose intolerance (Ishak 1986;

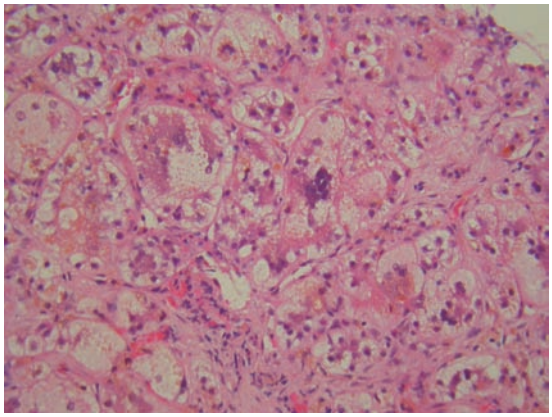

Figure 19.10. Neonatal hepatitis. Giant cell transformation of hepatocytes, cholestasis, and extra medullary hemopoiesis. This is not a discrete entity but an umbrella term for multiple different etiologies. (H&E, original magnification ×400.)

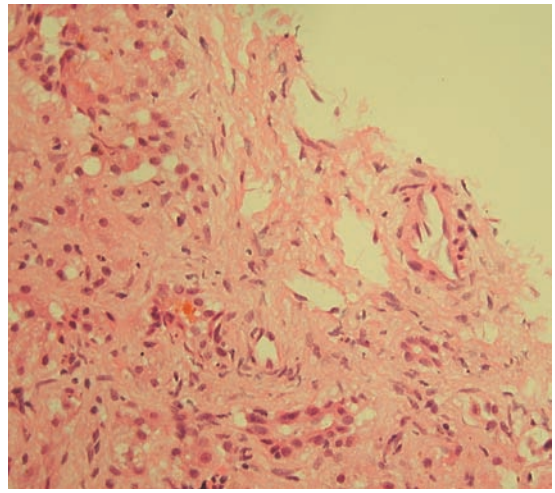

Figure 19.12. Galactosemia. Portal tract with a plug of orange bile in a ductule. (H&E, original magnification ×200.)

Jevon and Dimmick 1998b) (Fig. 19.11). Galactosemia can be accompanied by a degree of portal marginal ductular transformation, any bile plugs having a slightly orange hue (Fig. 19.12). Tyrosinemia and to a lesser extent galactosemia can feature marked siderosis. Excessive iron can also be a clue to the primary peroxisomal disorder of Zellweger's cerebrohepatorenal syndrome in the appropriate clinical setting (Jevon and Dimmick 1998a). If there is a history of splenomegaly Niemann-Pick type C disease is a possibility. Ultrastructural examination may reveal numerous myelin figures. This is an example of the utility of a liver sample in electron microscopy (EM) fixative. The clinical staff should be encouraged to submit tissue in this fixative. Tissue should also be submitted fresh or snap frozen for biochemical studies. Myelin figures feature thick bands of myelin, not to be confused with the fine filamentous whorled structure that bile can sometimes adopt (Figs. 19.13 and 19.14). The storage cells seen in relief on PAS staining in older chil-

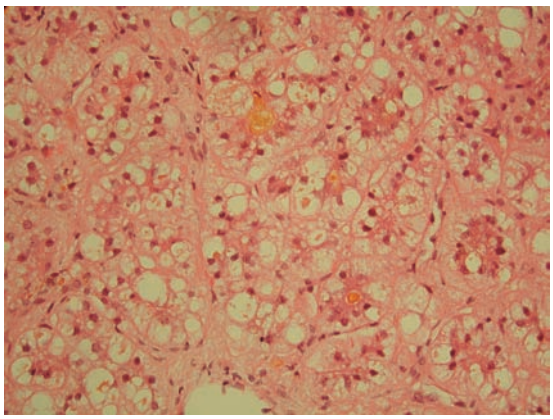

Figure 19.11. Galactosemia. Pseudoglandular cholestasis and steatosis. This pattern is also seen in tyrosinemia and hereditary fructose intolerance. (H&E, original magnification ×200.)

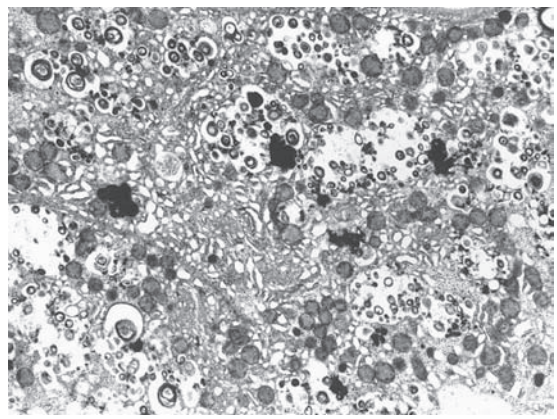

Figure 19.13. Niemann-Pick type C. Electron micrograph showing numerous round myelin figures. The myelin is thick and electron dense.

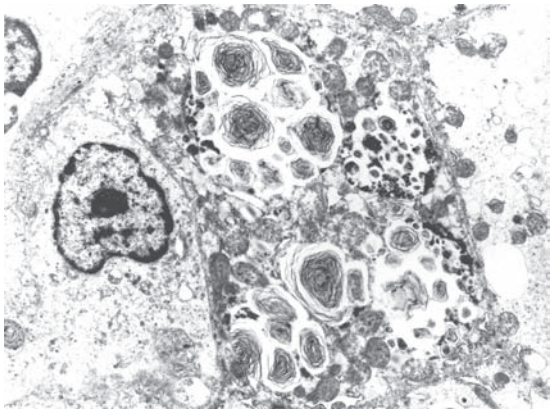

Figure 19.14. Bile. Electron micrograph showing fine filamentous whorled bile for comparison with Figure 19.13.

dren with Niemann-Pick disease are not identifiable in the neonatal period (Jevon and Dimmick 1998a). Cystic fibrosis (CF) can cause cholestasis and giant cell change in infancy, which can be accompanied by portal fibrous expansion and marginal ductular transformation. The eosinophilic inspissated material characteristic of CF is not seen before 8 months of age. Steatosis may be present and can be periportal. The biliary features can mimic EHBA (Shapira et al. 1999).

Clues to a Viral Etiology

Viral inclusions should be sought in biopsies showing an NNH pattern, but they are rarely seen. Immunohistochemistry can be helpful in selected circumstances. In symptomatic CMV infection, NNH may be accompanied by inclusions in biliary epithelium or, less frequently, hepatocytes. Bile duct inflammation induced by CMV in utero has been cited as a cause of paucity of intrahepatic bile ducts observed in infancy (Finegold and Carpenter 1982; Kage et al. 1993). Paramyxovirus-like inclusions have been described on ultrastructural examination in cases of NNH with large syncytial giant cells (Phillips et al. 1991; Hicks et al. 2001). However, giant cells, whatever their size, are a nonspecific finding (Lau et al. 1992). Rubella infection is a rare cause of NNH. The viruses described in the next section, which are usually associated with the clinical picture of fulminant hepatic failure, may also occasionally cause NNH rather than submassive necrosis in milder disease.

Treatment/Prognosis

Treatment and prognosis are determined by the specific etiology, if it is identifiable. Many cases are idiopathic and there is often spontaneous recovery.

Paucity of Intrahepatic Bile Ducts

Etiology/Pathogenesis

These conditions are grouped into syndromic and nonsyndromic forms. The syndromic group, Alagille syndrome or arteriohepatic dysplasia, is characterized by cholestasis with variable additional features, namely, posterior embryotoxon, butterfly-like vertebral arch defects, triangular facies, and peripheral pulmonary artery hypoplasia, either isolated or associated with complex cardiovascular abnormalities (Alagille et al. 1987). Alagille syndrome is an autosomal dominant condition with complete penetrance but variable expression. It is caused by mutations in the *JAG1* gene, a ligand for the Notch signaling pathway (Flynn et al. 2004).

The nonsyndromic group represents a wide variety of conditions and in many cases the cause remains unknown. A_1AT deficiency and CMV infection have been cited as causes along with the cerebrohepatorenal syndrome of Zellweger, coprostanic acidemia, and cystic fibrosis (Jevon and Dimmick 1998a). Down syndrome, other trisomies, hypopituitarism, syndromes associated with DPM, mitochondrial DNA depletion, and Niemann-Pick type C disease have also been described as causes albeit in small numbers (Kahn et al. 1986; Yehezkely-Schildkraut et al. 2003).

Histological Findings

The portal tracts lack an appropriately sized bile duct, that is, a duct of similar caliber to, and in close proximity to, the hepatic artery branch. It is always important to distinguish the bile duct proper from marginal ductules (Fig. 19.15). In normal subjects the ratio of bile ducts to portal tracts is 0.9:1.8. Paucity is said to be present if the ratio is 0.0:0.4 (Alagille et al. 1987). In practice, however, this is not an easy diagnosis to make. To calculate the ratio, at least 10 portal tracts are necessary (Hadchouel 1992), which is rarely

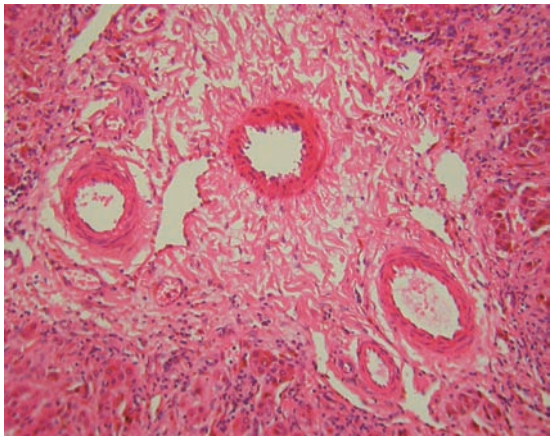

Figure 19.15. Paucity of bile ducts. Several hepatic artery branches are present, but there is no bile duct of a similar caliber. (H&E, original magnification ×200.)

achievable in neonatal biopsies, and the number of portal tracts themselves may be reduced in Alagille syndrome (Hadchouel et al. 1978). Another confounding factor is the gestational age of the patient: as the most peripheral portal tracts are the last to undergo ductal plate remodeling, an apparent paucity may be seen in biopsies from premature neonates, or even close to term. In some conditions the bile duct paucity evolves after birth and therefore may not be evident in an early biopsy. It has also been reported that in early Alagille syndrome biopsies there can be marginal ductular transformation complicating the differential with EHBA (Deutsch et al. 2001). Marginal ductules are not typical of later biopsies. Finally, studies of livers removed at transplantation in Alagille syndrome have shown that duct paucity is not uniform throughout the liver, and sampling error is therefore an issue (Libbrecht et al. 2005). Often the pathologist can only report an impression of paucity, and clinical correlation is required. The parenchyma is usually cholestatic with no other distinguishing features.

Treatment/Prognosis

In Alagille syndrome, management and outlook is determined by the constellation of multisystem disease. The majority of patients follow a benign course, but some patients suffer from pruritus, which may be intractable. Fibrosis is uncommon, but some patients require transplantation. In nonsyndromic cases, the outcome depends on the etiology, if known (Roberts 2004).

Rare Causes of Neonatal Jaundice

There are many causes of neonatal jaundice including endocrine disturbances and chromosomal aberrations. Of those who come to biopsy, children who have jaundice with a normal or low γ-glutamyltransferase level may have a bile acid synthesis disorder or a form of progressive familial intrahepatic cholestasis (PFIC); PFIC type 1 (Byler's disease) shows a bland cholestasis and characteristic coarse bile on EM, and PFIC type 2 has a pattern of NNH. These disorders have now been genetically defined (Jansen and Muller 2000; Knisely 2000).

The Neonate with Liver Failure

Liver failure can be secondary to a systemic process of multiorgan failure, for example, in circulatory collapse following severe hemorrhage, or it can be the initiator of a multisystem disorder. Hypotonia, hypoglycemia, coagulopathy, hyperammonemia, encephalopathy, renal tubular acidosis, and lactic acidosis are common findings. Sometimes liver failure presents initially simply as feeding difficulties with jaundice as a late feature (McClean and Davison 2003).

Clinical priorities beyond stabilizing the patient include establishing the etiology and the presence or absence of extrahepatic involvement to determine the appropriateness of transplantation (McKiernan 2004). This is a difficult decision, as age under 1 year at transplant is an adverse prognostic variable in pediatric fulminant hepatic failure of all causes (Baliga et al. 2004). Predicted timing of onset can assist in determining etiology. Cases with an intrauterine onset implicate neonatal hemochromatosis, congenital infection, and some metabolic (e.g., mitochondrial) conditions. Those presenting in the perinatal period could again be suffering from infection; toxic causes and metabolic conditions unmasked by the onset of feeding are also possibilities (McClean and Davison 2003). A French study of 80 infants with acute liver failure identified a metabolic etiology

in 42.5% (much higher than in other age groups), neonatal hemochromatosis in 16.2%, acute viral hepatitis in 15%, miscellaneous conditions (including neoplastic disease and paracetamol overdose) in 10%, and unknown etiology in 16.2% (Durand et al. 2001).

Determining etiology from histological findings in this scenario is often difficult, as the liver can show similar patterns of damage irrespective of cause, but clues to direct further investigation can sometimes be elucidated. In the above study, biopsy preceding transplantation or death was contributory to diagnosis in 18 of 26 cases (Durand et al. 2001). Viral, toxic, ischemic, and some metabolic conditions can cause necrosis, affecting the whole liver (massive) or, more often, leaving small areas of viable tissue (submassive). The necrosis usually affects entire lobules, but occasionally a zonal pattern can be identified. Perivenular and midzonal necrosis is typical of certain toxins (e.g., paracetamol). Hypovolemic states and cardiac failure also cause perivenular necrosis, the perivenular region being most vulnerable to hypoxia. In some metabolic conditions where the damage is subcellular, there is no necrosis and histological findings can be subtle.

Metabolic Causes of Liver Failure

In attempting to classify metabolic disease, authors have grouped conditions by clinical presentation (Portmann 1987) or by histological features (Ishak 1986; Jevon and Dimmick 1998a,b). Both approaches are useful to the pathologist, although metabolic conditions with well-described histological features may not demonstrate these conditions in the strict neonatal period. This has been alluded to in the previous section, where the DPAS-positive globules of A_1AT deficiency, the commonest metabolic disease seen in biopsy practice, cannot be expected in the neonate.

Histology in metabolic diseases can range from a picture of submassive hepatic necrosis (as described below for neonatal hemochromatosis), an appearance within the broad spectrum of neonatal hepatitis (these conditions overlap with those described in the previous section) or with surprisingly little morphological disturbance to the liver morphology. Where histological clues to etiology are known they are outlined below under each category of disease. Clinical correlation is essential in reaching a diagnosis.

Mitochondrial Respiratory Chain Disorders

Mitochondrial diseases are among the commonest metabolic causes of neonatal liver dysfunction. They are multisystem diseases with highly variable phenotypes. Renal, CNS, skeletal muscle, and cardiac involvement can be seen, among others. They can present with hepatomegaly or neonatal cholestasis as well as acute liver failure. Fetal ascites is sometimes a feature (Jevon and Dimmick 1998b). Raised plasma lactate is usual, but levels can fluctuate.

Dysfunction of the electron transport chain, made up of proteins encoded by both nuclear and mitochondrial DNA, leads to adenosine triphosphate (ATP) depletion and accumulation of free radicals. In cases of liver failure, deficiency of electron transport chain enzymes most frequently involves complexes I and IV. Deficiency of complex III can also cause liver failure, multiple complexes can be deficient or in the rare mitochondrial DNA depletion syndrome nuclear proteins essential for mitochondrial DNA replication are mutated, leading to DNA depletion (McClean and Davison 2003).

Histologically, in viable areas, there is steatosis, which can be predominantly microvesicular, along with cholestasis and sometimes giant cells. In those who survive the acute phase, fibrosis can occur later in the course of the disease (Jevon and Dimmick 1998b). Increased numbers of mitochondria may be identifiable as oncocytic hepatocytes or by EM where mitochondria may reveal abnormalities of shape and internal structure (Bioulac-Sage et al. 1993). Ultrastructural morphology is not diagnostic of specific mitochondrial diseases but suggestive appearances prompt measurement of the enzymatic activity of the respiratory chain complexes in affected tissues (Chow and Thorburn 2000; McClean and Davison 2003). In the rare cases where disease is apparently confined to the liver, transplantation may be appropriate but patients may succumb to extrahepatic disease, which only becomes manifest posttransplant (Morris 1999).

Disorders of Fatty Acid Oxidation

Fatty acids are an important energy source in periods of fasting via production of ketone bodies, especially in neonates where glycogen stores are low. In the liver the end product of fat metabolism is acetyl coenzyme A, which is produced in mitochondria following β-oxidation and is necessary for the production of ketone bodies. Babies therefore present with nonketotic hypoglycemia, sudden death, or a Reye's-like syndrome. Fatty acid oxidation disorders can follow illness in pregnancy (acute fatty liver or HELLP syndrome: hemolysis, elevated liver enzymes, and low platelet count).

Histologically, steatosis is the dominant feature, which can be mixed macro- and microvesicular or exclusively microvesicular or Reye's like. Reye's syndrome may often represent the unmasking of a metabolic defect rather than being a specific disease or toxic reaction (Glasgow and Middleton 2001). Canalicular cholestasis can also be a feature, sometimes with pronounced rosetting (Fig. 19.16).

Urea Cycle Defects

Urea cycle defects present acutely in the neonatal period. Four of the urea cycle enzymes can be defective, leading to accumulation of metabolites, ammonia, and glutamine, and hence to neurotoxicity. The four are carbamyl phosphate synthetase, ornithine transcarbamylase (OTC), arginosuccinic acid synthetase (citrullinemia), and arginosuccinate lyase. Ornithine transcarbamylase is X linked, and the other disorders are autosomal recessive. Histology is often surprisingly unremarkable with a little focal steatosis and very mild fibrosis. Aggregates of cleared hepatocytes containing glycogen have been described (Badizadegan and Perez-Atayde 1997; Jaffe 1998) (Fig. 19.17).

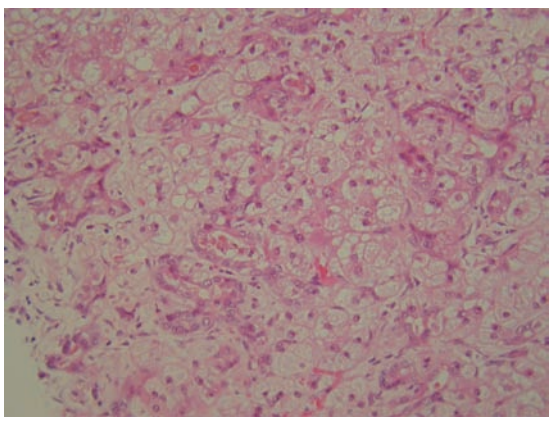

Figure 19.16. Fatty acid oxidation disorder. Acylcarnitine translocase deficiency. Parenchyma shows microvesicular steatosis and pseudoglandular cholestasis. (H&E, original magnification ×200.)

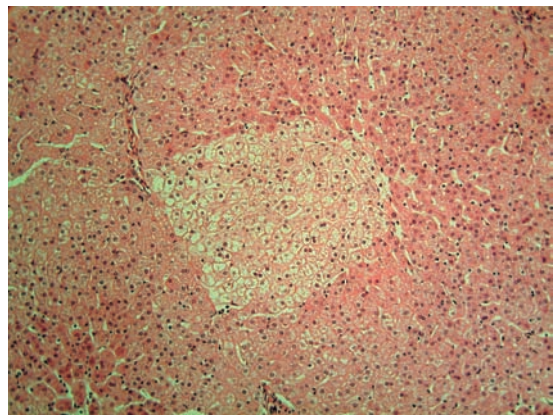

Figure 19.17. Carbamyl phosphate synthetase deficiency. Histological abnormalities are subtle. A cluster of clear glycogenated hepatocytes is demonstrated. (H&E, original magnification ×100.)

Organic Acidemias

These acidemias cause illness early in life, which is manifest as metabolic acidosis. Propionic acidemia and methylmalonic acidemia are examples; both have a spectrum of severity. They can be life threatening early in life or more insidious, and patients are vulnerable to infection (McKiernan 2004). Histological findings are unremarkable with only mild steatosis.

Galactosemia

This autosomal recessive disorder may present with liver failure very early in life, but occasionally presentation is slightly delayed. Presentation can be complicated by gram-negative sepsis. It is caused by a deficiency of galactose-1-phosphate uridyl transferase. Diagnosis is usually established by other means than biopsy, but when seen there is a distinctive pattern, shared by tyrosinemia and hereditary fructose intolerance cases, as described

above. Survivors are prone to significant liver fibrosis.

In the rare event that the diagnosis is missed, the liver at autopsy would show a similar pattern to the biopsy in viable areas with the addition of areas of necrosis/collapse (Ishak 1986; Portmann 1987).

Tyrosinemia

Tyrosinemia type 1 features a deficiency of an enzyme needed for terminal metabolism of tyrosine, fumaryl acetoacetate hydrolase, which leads to accumulation of toxic metabolites. Presentation is variable, but in the first 6 months of life the mode is usually liver failure. Initial histology resembles that seen in galactosemia, but regenerative nodules are an early feature containing more fat and less hemosiderin than the adjacent collapsed parenchyma. Later fibrosis develops, and a high risk of hepatocellular carcinoma persists even with the good results now achieved with dietary restriction and NTBC (2-2-nitro-4-trifluoromethylbenzoyl-1-3-cyclohexanedione, a bleaching herbicide which blocks toxic metabolites) (Holme and Lindstedt 2000).

Hereditary Fructose Intolerance

In hereditary fructose intolerance, a deficiency of aldolase B leads to accumulation of fructose-1-phosphate, sequestration of phosphate, and ATP depletion (Jevon and Dimmick 1998b). Histology again resembles galactosemia in the early stages, but is followed by only mild fibrosis.

Storage Disorders

These disorders rarely present in the neonatal period. The more usual presentation is with hepatomegaly, growth restriction, and variable degrees of hepatic and neurological dysfunction in the older child (Portmann 1987). In terms of acute presentations, however, lysosomal storage diseases are in the differential diagnosis of fetal ascites (McKiernan 2004). Of the glycogen storage diseases (GSD), severe forms of types II and IV as well as type I can present in the neonatal period. The liver manifestations, however, may be relatively mild, consisting of hepatomegaly. In type IV glycogen storage disease (GSD), DPAS-positive amylopectin-like material can be detected in the liver and other organs including the placenta. Type II has distinctive monoparticulate glycogen in lysosomes on ultrastructural examination. Type Ia can be diagnosed by the absence of staining with enzyme histochemistry for glucose-6-phosphatase on fresh tissue. Most GSD presents in the older child and with improvements in biochemical analysis biopsy is less frequently performed for diagnostic purposes. Niemann-Pick type C disease can present with cholestasis in the neonate and is discussed above. Wolman's disease, a deficiency of lysosomal acid esterase, can present acutely in the first few weeks of life. It gives rise to a macroscopically yellow/orange liver with histological fine microvesiculation of hepatocytes and Kupffer cells. Cholesterol crystals may be demonstrable in frozen sections. Pericellular fibrosis (common in several storage diseases) and portal fibrosis can ensue (Portmann 1987).

Neonatal Hemochromatosis

In contrast to adult-onset genetic hemochromatosis, the pattern in a neonate of multiorgan parenchymal iron storage with sparing of the reticuloendothelial system, termed neonatal hemochromatosis (NH), is not currently defined by specific genetic abnormalities (Kelly et al. 2001). It is best regarded as a syndrome, a secondary manifestation of intrauterine liver failure rather than a specific defect of iron handling, and there may be multiple etiologies. There is usually intrauterine growth restriction (IUGR) and often prematurity. Neonates present early with liver failure. Investigations are likely to include a lip biopsy, which needs to include salivary gland tissue to determine the presence or absence of iron within parenchymal cells (not macrophages). This can be a sensitive diagnostic technique in patients who often are suffering from a coagulopathy complicating potential liver biopsy (Smith et al. 2004) (Figs. 19.18 and 19.19). Magnetic resonance imaging (MRI) to look for parenchymal iron storage in other organs (e.g., the pancreas) might also be helpful.

Etiology/Pathogenesis

Neonatal hemochromatosis is known to recur in siblings. Occurrence with consanguineous parents

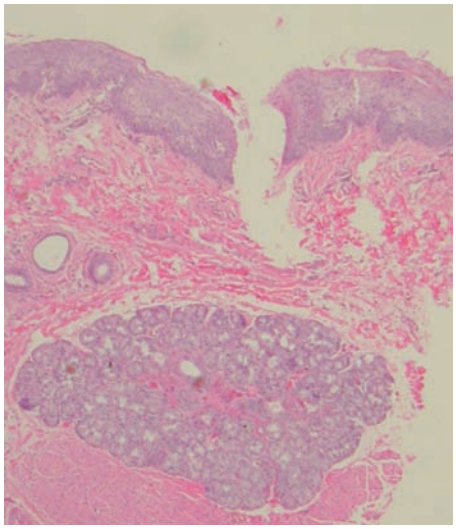

Figure 19.18. Lip biopsy in neonatal hemochromatosis. This is an adequate biopsy as salivary gland tissue is included. (H&E, original magnification ×40.)

has been observed, implicating an autosomal recessive inheritance, but maternal factors seem important as recurrence occurs even with different fathers (Knisely et al. 2003). An immune mechanism has been postulated and supported by results of immune intervention in subsequent pregnancy (Kelly et al. 2001; Whitington and Hibbard 2004). That iron handling itself is not defective is supported by the fact that iron accumulation does not seem to recur in survivors with the exception of one case report posttransplantation (Egawa et al. 1996).

Histological Findings

The pathologist may be confronted with a biopsy or with the whole liver, which is usually small with a wrinkled capsule, removed at transplant or seen at autopsy. The picture is usually that of submassive hepatic necrosis; that is, only small nodules of parenchyma survive, with the remaining liver tissue showing necrosis and collapse (Fig. 19.20). This does give a nodular architecture to the liver, but this should not be immediately interpreted as cirrhosis. Connective tissue stains are essential in the assessment of architecture, which is much more likely to show collapse between nodules, outlined by reticulin and pale staining on Masson trichrome, rather than the mature elastin-rich septa of cirrhosis (orcein stain) (Figs. 19.21 and 19.22). In longer-standing cases mature fibrous tissue will be laid down. Ductular transformation is often florid, as is always seen in areas of collapse. This should not be mistaken for evidence of a biliary process, as the portal tracts are normal. Surviving parenchyma may show cholestasis and giant cell change with extramedullary hemopoiesis. Perls staining reveals iron deposition in

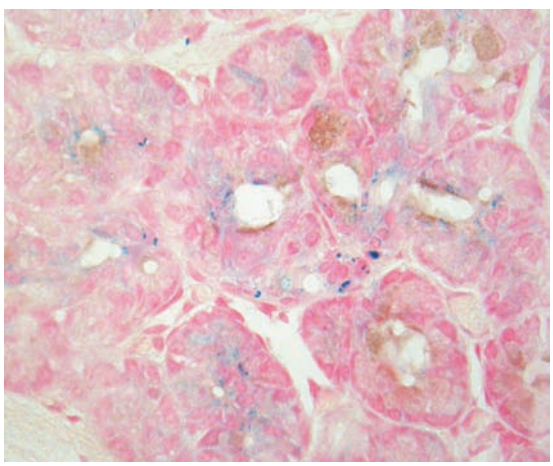

Figure 19.19. Lip biopsy in neonatal hemochromatosis. Perls stain to demonstrate iron deposition in parenchymal cells. (Perls, original magnification ×400.)

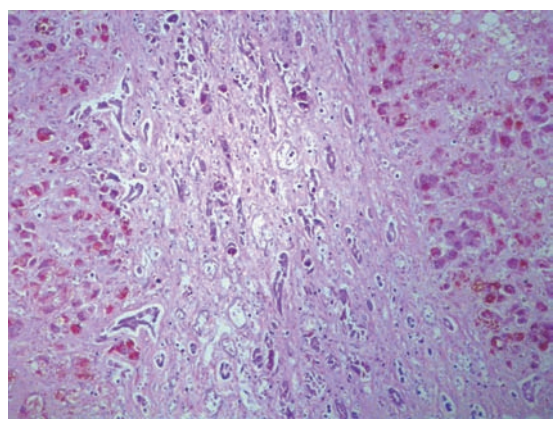

Figure 19.20. Neonatal hemochromatosis. Submassive hepatic necrosis. Collapsed areas between surviving nodules show ductular transformation. A similar pattern is seen in any cause of submassive necrosis, viral, toxic or metabolic. (H&E, original magnification ×200.)

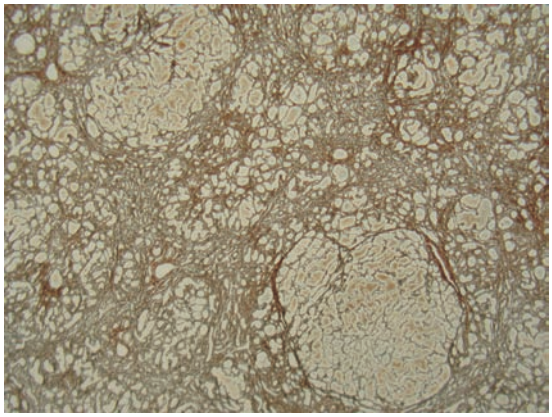

Figure 19.21. Neonatal hemochromatosis. Submassive hepatic necrosis. Reticulin stain outlines the collapse, which delineates the nodules. (Reticulin, original magnification ×40.)

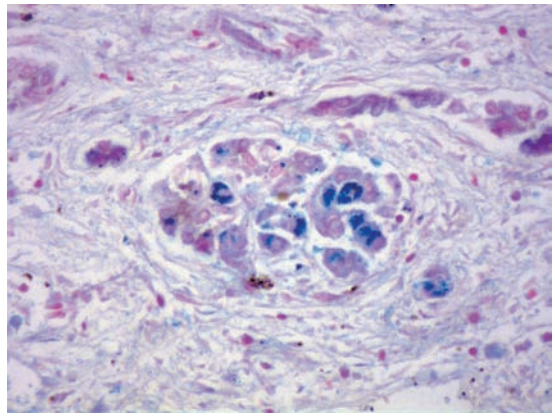

Figure 19.23. Neonatal hemochromatosis. Perls stain demonstrates iron in parenchymal cells. This is not diagnostic in isolation. (Perls, original magnification ×400.)

hepatocytes and ductular cells in excess of that in macrophages (Moerman et al. 1990) (Fig. 19.23). It should be noted that iron deposition is not uncommon in any cause of submassive hepatic necrosis and is a positive feature of several metabolic disorders (tyrosinemia, Zellweger syndrome). Although the iron is described as being more conspicuous in parenchymal cells than macrophages, liver findings in isolation cannot be relied upon for definitive diagnosis without evidence of extrahepatic parenchymal iron deposition. Pancreas, thyroid, and kidneys are common sites at autopsy; macrophages in lymph nodes, spleen, and bone marrow are typically spared. Iron deposition in salivary gland cells is referred to above.

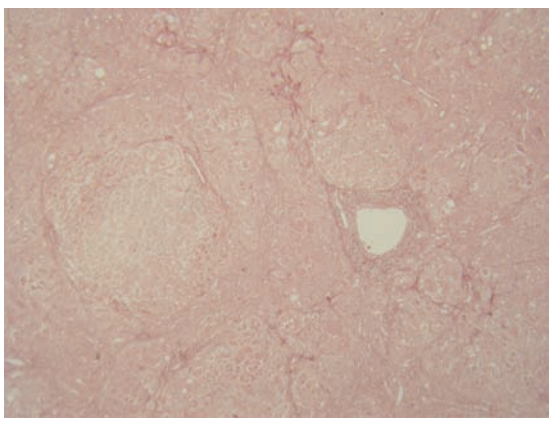

Figure 19.22. Neonatal hemochromatosis. Submassive hepatic necrosis. Orcein stain shows that elastin (black), which defines mature fibrous tissue, is limited to a portal tract and is not seen around nodules. This therefore represents acute damage rather than cirrhosis. (Orcein, original magnification ×40.)

Treatment/Prognosis

The outlook for these infants has been poor. They often die in the first month. Management is supportive. An antioxidant cocktail, if instituted early in life, has been observed to increase survival without subsequent reaccumulation of iron. Results of transplantation are not as good as in other indications, but again survival has been observed and early transplant assessment is recommended (Flynn et al. 2003). Neonatal hemochromatosis is one of the commonest indications for neonatal transplant assessment. After one affected pregnancy, results of high-dose intravenous immunoglobulin given in a subsequent pregnancy can alter the severity of recurrence with greatly improved neonatal outcome (Whitington and Hibbard 2004).

Infectious Causes of Liver Failure

The liver is the first organ to receive blood from the placenta and therefore it is vulnerable to transplacental infections. It is also vulnerable to pathogens introduced via umbilical catheters in the neonate. Infections in the neonate are often manifest as a systemic illness. Some cause liver

failure as a major manifestation; in others the hepatic disease is of less importance than involvement of other organs. Sometimes there is a neonatal hepatitis secondary to infection rather than a picture of fulminant hepatic failure, and there is therefore some overlap with the previous section on the jaundiced neonate. In severe disease the liver shows a picture of submassive hepatic necrosis as described above in the neonatal hemochromatosis section. Where specific clues to an infectious etiology have been described they are outlined below.

Viruses

Enteroviruses

Enteroviruses are most prevalent in late summer and autumn when they cause a spectrum of illnesses. In the neonate, who is particularly vulnerable, overwhelming infection that involves the liver is most likely to be due to echovirus infection. This is often accompanied by a systemic picture of disseminated intravascular coagulation (DIC). Echovirus infection produces a picture of intensely hemorrhagic perivenular necrosis in the early stages with endotheliitis, fibrin thrombi, and fibrinoid necrosis of hepatic arteries and veins. Later in the course of the infection dystrophic calcification (common to many long-standing viral infections) and veno-occlusive lesions are features. These appearances have prompted speculation that the echovirus exerts a detrimental effect on endothelial cells early in the course of the disease. Viral inclusions are not seen (Wang et al. 2001). Coxsackie viruses can cause a neonatal hepatitis, but hepatic manifestations are usually overshadowed by cardiac and meningeal involvement.

Herpes Viruses

Herpes simplex virus (HSV) infection, usually acquired by exposure to maternal genital secretions or lesions at delivery, can cause liver failure in isolation or a multisystem disorder. Histologically there is severe submassive hepatic necrosis. Typical inclusions are usually seen at the margins of the necrotic areas (White and Dehner 2004) (Fig. 19.24). Inflammation is sparse. Immunohistochemistry is useful for confirmation. Cytomegalovirus is a common congenital infection but it is usually asymptomatic. It is a rare cause of acute liver failure. Cytomegalovirus is more likely to give a neonatal hepatitis picture as discussed above. Varicella infection can occur in the newborn and cause systemic disease, but is a rare cause of liver failure. Human herpes virus 6 is another rare cause of neonatal liver failure.

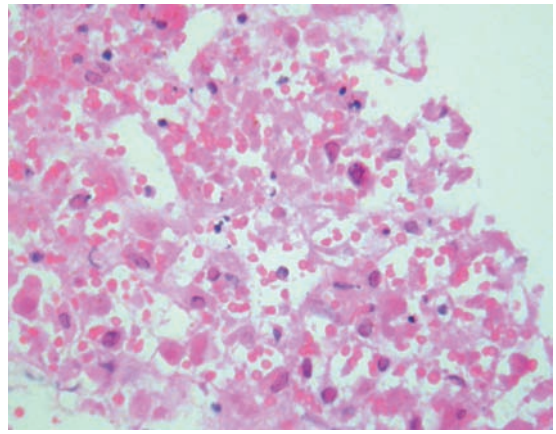

Figure 19.24. Herpes simplex virus inclusions. These are present in hepatocytes at the edge of an area of necrosis. (H&E, original magnification ×400.)

Adenovirus

Adenovirus can cause hepatic failure, but usually only in the immunocompromised host. Inclusions can be seen (Wang and Wang 2003; White and Dehner 2004).

Parvovirus B19

This virus is a cause of fetal hydrops. It infects erythroid precursors. Presentation can be with acute hepatic failure in association with aplastic anemia (Langnas et al. 1995). Siderosis and erythropoiesis can be conspicuous in the liver (White and Dehner 2004). Inclusions can be seen; they are also present in the placenta and other organs.

Hepatotropic Viruses

Hepatitis A, rare in the neonate, can cause a nonspecific diarrheal illness. Hepatitis A and E are only significant causes of hepatic failure in countries where they are endemic: India and parts of Africa (White and Dehner 2004). Hepatitis B is

rarely seen in countries where prenatal maternal screening and postnatal prophylaxis with hepatitis B immunoglobulin and hepatitis B immunization are the norm. This scheme avoids chronic disease (Durand et al. 2001). If this regimen is not instituted or fails, the infection is subclinical in the neonatal period but can cause acute hepatic failure at 3 to 4 months of age (McKiernan 2004). Hepatitis C can be vertically acquired. The risk is related to maternal viral load and HIV status, but infection is not manifest in the neonatal period; the virus does persist into childhood, however (Tovo et al. 2003).

Nonviral Infections

Systemic bacterial infections often cause jaundice and can lead to bile plugging in marginal portal ductules. A gram-negative septicemia can be an indicator of galactosemia. Bacterial infections introduced via an umbilical catheter cause small abscesses. In listeriosis, meningitis dominates the clinical picture, but small foci of necrosis can be seen in the liver (Dimmick and Jevon 1998). In perinatally acquired tuberculosis the clinical presentation can be atypical; foci of necrosis and later caseating granulomas would be seen in affected livers. The tubercle bacillus prefers the well-oxygenated left lobe in transplacentally acquired infection. Syphilis is now very rare but is a cause of liver failure. Toxoplasma may cause hepatitis, but liver manifestations are usually overshadowed by CNS involvement.

Trauma and Iatrogenic Conditions Including Total Parenteral Nutrition

Trauma

Although trauma has traditionally been implicated in the formation of subcapsular hematoma in the fetus and neonate, this has not been borne out in large studies (French and Waldstein 1982; Singer et al. 1999). The study by Singer et al. (1999) found subcapsular hematoma in 52 of 755 perinatal autopsies (6.9%). Significantly more subcapsular hematoma cases had sepsis than did the control group (62% vs. 25%), and the hematoma group also had more cerebral germinal matrix hemorrhages. The authors did not find any association between trauma, maternal conditions (e.g., preeclampsia), placental conditions (e.g., abruption) or fetal/neonatal conditions (e.g., coagulopathy), and hematoma formation. They therefore speculate that hematoma formation has more to do with the effects of leukocyte enzymes and inflammatory cytokines on the liver capsule than with trauma. They also point out that the low birth weight/premature neonate is less well equipped to cope with sepsis and germinal matrix hemorrhage than is the more mature neonate. Low birth weight infants are therefore more vulnerable to life-threatening complications such as hematoma rupture (French and Waldstein 1982; Foss 2004).

A rare cause of hemoperitoneum attributed to trauma in the newborn is rupture of the intra-abdominal umbilical vein (Miller et al. 1987). The umbilical vein can also be damaged by catheterization, a potential source of infection, vasospasm, and thromboembolic events (Hermansen and Hermansen 2005). Umbilical artery catheters are subject to similar complications. High placement of the catheter, above the diaphragm, is recommended to reduce the incidence of these events (Barrington 2000).

Biopsy

Needle liver biopsy is a valuable diagnostic technique in the neonate. Complications are infrequent. A study of 184 biopsies in patients under 1 year of age revealed no major complications or deaths, but a drop in hemoglobin was observed in three, transient hypotension in one, and hematoma at the biopsy site in one (Lichtman et al. 1987). For the pediatric population as a whole, the rate of major complications is cited as 1.7% if the biopsy site is assessed by ultrasound (Kader et al. 2003). Children with coagulopathy or cancer, or those who have undergone bone marrow transplantation are at increased risk (Cohen et al. 1992).

Drugs

Drug handling by the neonatal liver is influenced not only by the maturing nature of enzyme systems, which is felt to occur fairly rapidly, but

also by the dynamic vascular changes giving rise to alterations in blood flow, oxygenation, and the gradual loss of shunting via the ductus venosus (Gow et al. 2001). The incidence of adverse drug reaction in the neonate is estimated at 10%, a further complication being the lack of dosage guidelines specifically for the neonate (McIntyre and Choonara 2004). Histologically, drug reactions can mimic most disease processes in the liver and therefore are often a differential diagnostic consideration.

Total Parenteral Nutrition

Parenteral nutrition has allowed the survival of children with intestinal failure. This may be due to short gut, for example, following resections for necrotizing enterocolitis or gastroschisis, abnormalities of the mucosa such as microvillous inclusion disease, or dysmotility disorders/pseudo-obstruction. Unfortunately, TPN has significant complications including catheter sepsis, loss of venous access, metabolic disorders, and pulmonary embolism. The most serious complication is TPN-induced liver disease. Most vulnerable are neonates, especially those who are of low birth weight, small for gestational age, or premature, and in those in whom it is not possible to ever initiate enteral feeding (Baserga and Sola 2004).

Etiology/Pathogenesis

The mechanisms by which TPN damages the liver are not understood. Various components of the feeding solution have been postulated as deleterious, but there is no clear candidate. Possible contaminants, for example, of lipid emulsions by plant sterols (phytosterols), have been observed to reduce bile secretory capacity in animal studies (Iyer et al. 1998). Lack of certain nutrients has also been suggested but not substantiated. It is likely that the damage is multifactorial (Heine and Bines 2002).

Histology

Total parenteral nutrition liver disease is characterized by canalicular cholestasis and ballooned hepatocytes. Steatosis is rarely a feature in the neonate. As the duration of the TPN lengthens, progressive portal and pericellular fibrosis develop, accompanied by marginal bile ductular proliferation (Fig. 19.25). Mild fibrosis has been described in under 2 weeks of TPN and severe fibrosis has been described after only 12 weeks of TPN (Zambrano et al. 2004). Bile plugging can be seen in ductules, similar to that seen in large duct obstruction or systemic sepsis, which may be concurrent. Debris-laden macrophages are seen in portal and parenchymal areas. These can have a light tan color on hematoxylin and eosin (H&E) and stain strongly with DPAS. Extramedullary hemopoiesis and occasional giant cells may be present, which are nonspecific findings in the neonatal liver biopsy.

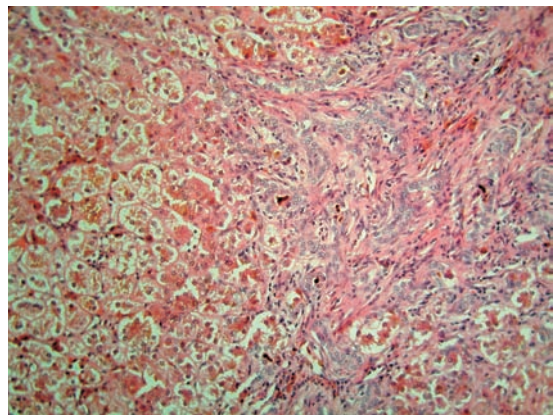

Figure 19.25. Total parenteral nutrition. An expanded portal tract shows biliary features including ductular bile plugs. Parenchymal hepatocytes show ballooning and cholestasis. (H&E, original magnification ×200.)

Treatment/Prognosis

In those with postsurgical short gut, if the remaining bowel is able to adapt and enteral feeding is successfully introduced, the effects on the liver can be stalled. Occasionally, an isolated liver transplant can prolong the time available for intestinal adaptation. Patients benefit from specialist nutritional care and early assessment for transplantation either of small bowel alone or, where liver fibrosis is advanced, from combined small bowel and liver transplantation (Kaufman et al. 2003). It should be noted that the allograft liver is vulnerable to the effects of TPN where it is continued in the posttransplant period,

complicating the assessment of allograft biopsies.

Tumors

The liver can be a site of metastasis in, for example, neuroblastoma, lymphoma, and Wilms' tumor. Primary liver tumors account for only 1% to 4% of solid tumors in children. Under 2 years of age the commonest primary tumors are hepatoblastoma, vascular lesions, and mesenchymal hamartoma. These will be discussed further. Tumors such as hepatocellular carcinoma, undifferentiated embryonal sarcoma, and focal nodular hyperplasia are tumors of older children, and other tumors, for example primary yolk sac tumor and embryonal rhabdomyosarcoma of the biliary tree, are very rare (Stocker 1998, 2001).

Hepatoblastoma

This is the most common primary hepatic neoplasm of children, and 90% occur before 5 years of age; 4% are present at birth. An increasing incidence among very low birth weight infants has been noted (Reynolds et al. 2004). Hepatoblastoma can be seen either as part of a syndrome; examples are Beckwith–Wiedemann syndrome and familial adenomatous polyposis, or as an isolated lesion. Presentation is usually with an enlarging abdomen; in older boys precocious puberty can be observed due to human chorionic gonadotrophin (hCG) production by the tumor. Thrombocytosis and anemia are frequent, but the most useful finding both in terms of diagnosis and for following response to treatment is of a grossly elevated AFP. It should be noted that an elevated AFP is normal in the neonatal period; other liver tumors have been associated with elevated AFP, for example, mesenchymal hamartoma (Boman et al. 2004), and poorly differentiated hepatoblastoma can have a normal AFP. The tumor is usually a single mass, more often in the right lobe, but can be multifocal. Where primary resection is not possible, preoperative chemotherapy is given to down-stage the tumor prior to removal or to transplantation. The lungs are a favored site of metastasis (Stocker 2001).

Pathological classification divides tumors into epithelial and mixed epithelial and mesenchymal types:

1. Epithelial type: fetal, embryonal, macrotrabecular and small cell undifferentiated
2. Mixed epithelial and mesenchymal: with mesenchymal tissue limited to osteoid, cartilaginous, and fibrous tissue; in the teratoid variant, other elements are present (e.g., striated muscle, squamous epithelium, melanin) (Stocker 2001)

In the epithelial categories fetal cells are well differentiated but smaller than adjacent hepatocytes (Fig. 19.26). The cells form slender cords with intercellular canaliculi, which may contain bile. The tumor cells can be clear due to the presence of glycogen or fat, but no portal structures are present within the tumor. Embryonal cells have a higher nuclear to cytoplasmic ratio, more prominent nucleoli, and more frequent mitoses (Fig. 19.27). Transition between fetal and more cellular embryonal areas is common. In the macrotrabecular type there is an architectural arrangement of trabeculae 10 to 20 cells thick; the constituent cells can be fetal or embryonal but are occasionally large. The presence of typical areas of hepatoblastoma elsewhere in the lesion allows differentiation from hepatocellular carcinoma. The small cell undifferentiated variant features sheets of round, oval, or spindled small cells with a high mitotic rate (Haas et al. 1989). The influence of histological type on prognosis has been debated, but completely resected tumors with

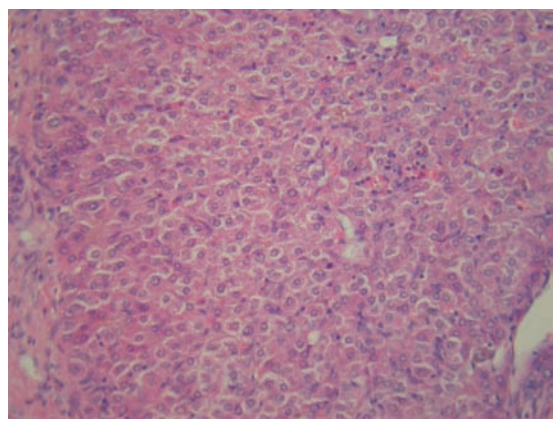

Figure 19.26. Hepatoblastoma. Fetal type cells. (H&E, original magnification ×200.)

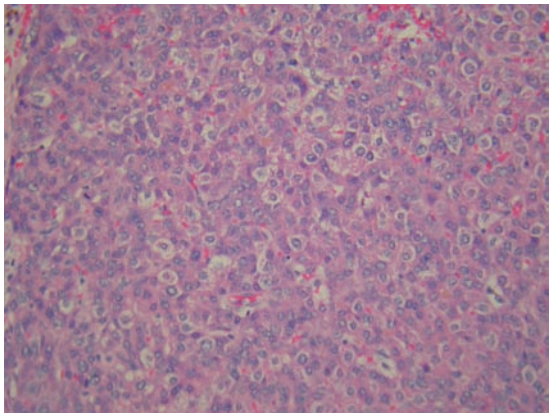

Figure 19.27. Hepatoblastoma. Embryonal type cells. (H&E, original magnification ×200.)

pure fetal histology have a superior prognosis (Haas et al. 1989). Determination of subtype in a biopsy will be influenced by sampling error and pretreatment (Rowland 2002; Boman et al. 2004). Other postulated prognostic variables have included gains on chromosomes 8q and 20 and nuclear staining for β-catenin (a protein involved in cell adhesion that acts as a positive growth signal when translocated to the nucleus) as predictors of poor outcome (Weber et al. 2000; Park et al. 2001). In some treatment protocols the pathologist may see the tumor only in a resection specimen postchemotherapy, which may induce considerable changes. Necrosis is extensive in sensitive tumors, and osteoid is typically more conspicuous postchemotherapy (Saxena et al. 1993) (Fig. 19.28).

Hepatic Vascular Lesions

Vascular lesions of the liver are the most common lesion seen in the first 6 months of life. The nomenclature has been inconsistent and confusing. The use however of GLUT-1 (an erythrocyte glucose transporter) immunohistochemistry has led the way to the identification of two distinct hepatic vascular lesions of infancy—one a neoplasm and the other a malformation. The first, termed hepatic infantile hemangioma (HIH) by Mo et al. (2004), incorporates lesions previously referred to as infantile hemangioendothelioma (of both types as described by Dehner and Ishak 1971) or cellular or juvenile hemangioma. This is usually asymptomatic, presenting as an incidental finding or asymptomatic hepatomegaly in the first few weeks or months of life. It comprises multiple well-defined nodules, microscopically consisting of closely packed proliferating capillaries with variable central involution. There is consistent GLUT-1 positivity in endothelial cells. It is responsive to steroid or interferon treatment and shows spontaneous regression. These features are common to this lesion and to cutaneous juvenile hemangioma, with which it can coexist, leading to the hypothesis that these two lesions are biologically identical. Caution should be exercised in nonregressing lesions presenting in older children as angiosarcoma arising in HIH has been described.

The second lesion is a hepatic vascular malformation termed hepatic vascular malformation with capillary proliferation (HVMCP) (Mo et al. 2004). This has been previously described as hepatic cavernous hemangioma, solitary hemangioma, and arteriovenous malformation. In contrast to HIH, this is symptomatic at or near to birth, with cardiomegaly, cardiac failure, anemia, edema/hydrops, or consumptive coagulopathy (Kasabach-Merritt syndrome) (Prokurat et al. 2002). Again, in contrast to HIH, this forms a single large mass that merges with the surrounding liver microscopically. Large atypical vessels are present and there is often hemorrhage, infarction, and calcification. Closely packed

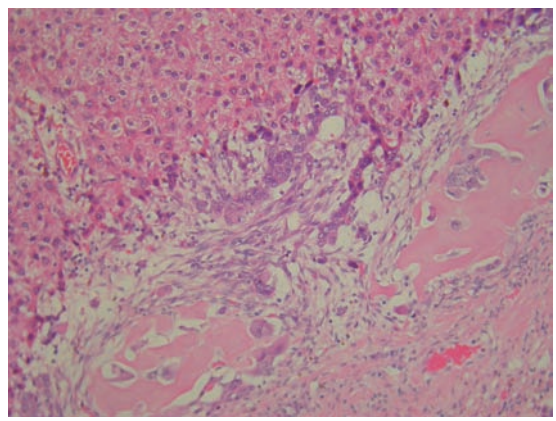

Figure 19.28. Hepatoblastoma. Osteoid, which is often more conspicuous in postchemotherapy samples. (H&E, original magnification ×200.)

capillaries can be seen at the periphery, which may be resemble HIH, but GLUT-1 is consistently negative. Response to steroids or interferon is unlikely, and surgical resection is required to control the cardiovascular and hematological complications.

Mesenchymal Hamartoma

This is a lesion of young children, 85% occurring before 2 years of age. It presents as an enlarging mass in the liver, the enlargement due to the accumulation of fluid in cysts (Stocker 2001). Rarely, serum AFP can be elevated (Boman et al. 2004). The mass is more common in the right lobe and may be pedunculated. Histologically the lesions consist of a myxoid matrix containing biliary structures, which can be distorted, or even ductal plate–like collagen bundles and occasional cords of hepatocytes. The cysts, if present, may lack a lining epithelium (Stocker 2001). Mesenchymal hamartoma has been regarded as a developmental anomaly; however, it may be etiologically heterogeneous with some lesions representing neoplasms (Bove et al. 1998). A translocation involving the long arm of chromosome 19 has been described as has an association with the development of undifferentiated embryonal sarcoma (UES) (O'Sullivan et al. 2001). The latter scenario has led to the hypothesis that mesenchymal hamartoma may be the precursor of some cases of UES. While spontaneous resolution has been described (Barnhart et al. 1997), in view of the above, complete excision and thorough sampling is prudent (O'Sullivan et al. 2001).

Gallbladder

Developmental anomalies of the gallbladder include absence, hypoplasia, and septation (Nakazawa et al. 2004). An absent gallbladder is often accompanied by other malformations in other organ systems (Turkel et al. 1983). The gallbladder is often absent or abnormal in EHBA. Acquired lesions include marked distention in the septic neonate with colorless bile, empyema, and rarely cholelithiasis (Jamieson and Shaw 1975; Peevy and Wiseman 1982; Jeffrey et al. 1984).

Miscellaneous Conditions

Infants Born to Diabetic Mothers

Diabetes in mothers tends to result in large babies who have correspondingly large livers. Fetal liver volume as assessed by ultrasound is related to maternal glycemic control and can be used to identify growth acceleration (Boito et al. 2003). In the immediate postnatal period the neonate may be hyperinsulinemic and is therefore vulnerable to hypoglycemia, even where maternal glycemic control is good (Sunehag et al. 1997). Infants of diabetic mothers have augmented fetal hemoglobin synthesis and can be polycythemic at birth. Increased erythropoiesis has been observed in the liver and is associated with reduced liver iron concentrations secondary to the increased demand (Petry et al. 1992).

Down Syndrome

Children with Down syndrome have a 10- to 20-fold increased risk of developing leukemia, particularly of the megakaryoblastic type. This includes a transient form of neonatal megakaryoblastic leukemia. In most infants this is asymptomatic, and it may be associated with increased numbers of megakaryocytes being seen in the liver. In a portion of cases, however, the transient leukemia is severe and potentially life threatening, associated with hydrops, effusions, and liver or multiorgan failure. Those who achieve spontaneous remission are still at risk of developing leukemia in 13% to 33% of cases (Massey 2005). Cholelithiasis, while rare in all groups of children, is seen with an increased frequency in Down syndrome (Toscano et al. 2001). Paucity of bile ducts has also been described (Kahn et al. 1986; Yehezkely-Schildkraut et al. 2003).

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20
The Respiratory System

Steve J. Gould

One of the most critical events of birth is the conversion of the fluid-filled lung, unimportant to fetal intrauterine existence, into a hollow organ distended with air and capable of gaseous exchange sufficient to support life. Indeed, it has been argued that the major determinant of perinatal survival is respiratory function (Wigglesworth and Desai 1982).

The failure to make this conversion adequately may lead, directly or indirectly, to infant death, and the pathologist often needs to assess the contribution made by respiratory inadequacy to the sequence of events leading to death. In the preterm infant, problems are mainly related to pulmonary immaturity and associated therapy. In the mature infant, birth asphyxia primarily results in cerebral damage but can engender significant respiratory complications when associated with aspiration of meconium. Even in stillbirths, where primary pulmonary pathology is rarely a cause of death, lung pathology may provide clues to antecedent events. Poor lung growth and maturation may point to the presence of pathology elsewhere. Consequently, adequate pathological investigation of the fetal or infant respiratory system is critical in any perinatal autopsy.

Examination of the Respiratory System

Postmortem radiology may be useful in identifying pneumothoraces, although some caution is needed in interpretation of other pulmonary changes because of postmortem absorption of air. Where radiology is not available, the more conventional approach to diagnosing pneumothorax is to release pleural air under water. This can often be achieved easily by immersing the thorax under water.

Choanal patency can be tested by passing a probe through each of the nares in turn and ensuring it reaches the nasopharynx. Inspection of the mouth may reveal palatal clefts and whether they involve the hard or soft portions. The larynx should initially be examined for the presence of clefts and, in conjunction with dissection of the esophagus, tracheoesophageal fistulas. After removal of the esophagus, the form of the tracheal rings should be checked for either complete rings or rings with large posterior pars membranacea that might accompany tracheomalacia. My preference when examining for laryngeal anomalies, especially when suggested by the history or presence of pulmonary hyperplasia (vide infra), is to cut the larynx transversely into three or four blocks for step or serial histological sectioning (Gould and Howard 1985).

Lungs should be removed with the heart to allow inspection of the pulmonary arterial and venous system. The lower borders of the lungs and heart should be approximately at the same level; if they are not, it is often an indication of lung hypoplasia. Lung weights followed by calculation of lung body weight ratios are the simplest and most useful guide to the normality or abnormality of lung growth (see below). Inflating one of the lungs with formalin instilled through the airways can be valuable, particularly in assessing maturity. The precise diagnosis of lung pathology

macroscopically is difficult and histology is mandatory, with at least one block from each main lobe.

Normal Development

The respiratory system can be divided developmentally into upper and lower tracts. While histoanatomic discussion inevitably dominates the approach to respiratory development, it is important to consider the maturation of vital biochemical pathways, the immaturity of which contributes significantly to the postnatal morbidity and mortality of preterm infants.

Upper Respiratory Tract

The nose and mouth commence development at 5 weeks postconceptional age and are derived from five main facial processes: a fused pair of frontonasal prominences, and paired maxillary processes and paired mandibular processes. Migration of these processes, derived from cephalic neural crest, is a precisely orchestrated series of events and failure may lead to a wide variety of facial malformations.

The maxillary processes fuse with the frontonasal processes and their medial point of fusion becomes the philtrum of the upper lip. Caudally the mandibular processes fuse and the intervening space between mandibular and maxillary processes becomes the primitive mouth, or stomatodeum. On either side of the midline of the frontonasal process, thickening of the epithelium forms circular nasal disks or placodes, each of which recede from the surface due to a combination of active invagination and proliferation of surrounding mesenchyme. Eventually this burrowing activity forms the anterior nares (Ferguson 1991). The newly developed nasobuccal membrane separating the nose from the mouth breaks down posteriorly at 7 weeks to form the communicating posterior choanal space. Anteriorly the bucconasal membrane ultimately remains as the primary palate, the remainder being replaced by the secondary or definitive palate derived from horizontal palatine processes. Fusion commences at the primary palate and extends rostrally. Normal palatal fusion is dependent on epithelial programmed cell death (Goldman 1992) at the point of fusion and mesenchymally signaled epithelial differentiation (Ferguson et al. 1992; Rice et al. 2004).

The larynx develops separately but at the same time from the endodermal lining of the laryngotracheal tube and the mesenchyme of the fourth to sixth branchial arches. A diverticulum forms in the ventromedial aspect of the foregut at day 20, and gives rise to the larynx, trachea, and lungs. Separation of the larynx and trachea from the esophagus occurs by ingrowth of tissue to form the tracheoesophageal septum. During development, proliferation of mesenchyme in the lateral wall of the larynx partially obliterates the lumen for a time. Final recanalization occurs between the 8th and 10th week of gestation (Hamilton et al. 1972; Zaw-Tun 1988).

Lower Respiratory Tract

As described above, the trachea becomes separated from the esophagus by the tracheoesophageal septum. In the embryonic phase, the lung appears as a ventral outpouching of the endodermal foregut by the fourth week after ovulation. This forms the epithelial components, but branching begins within mesenchyme that forms the walls of the airways and blood vessels. Segmental airways are present by 6 weeks. Although lung development is divided into different stages by different authors, there is agreement as to the main events and timing (Burri 1999; Hislop 2003).

Pseudoglandular Phase (7 to 17 Weeks)

Major lung components develop including bronchial glands, cartilage, and, toward the end of this period, ciliated epithelium. The bronchial buds divide dichotomously with completion of preacinar branching by 17 weeks, although most divisions are complete by 14 weeks' gestation and faster in the right than the left lung. Airways terminate in blind ending tubes, and are lined by low columnar or cuboidal epithelium containing glycogen (Fig. 20.1). Mesenchyme is abundant, but capillaries are sparse and not closely apposed to epithelium. Respiration is not possible at this stage.

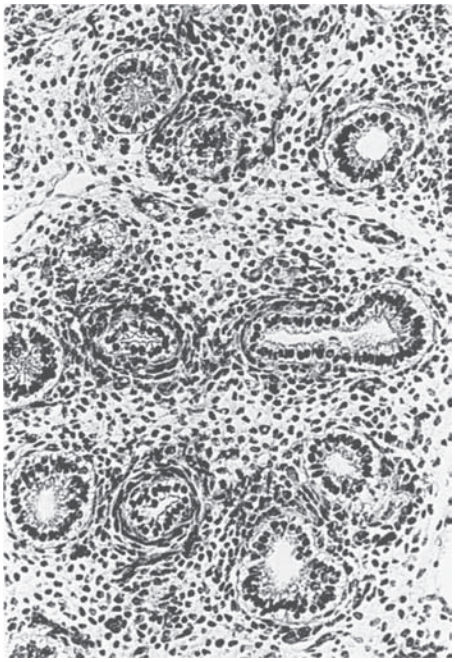


Figure 20.1. Lung from a 13-week spontaneous abortion showing pseudoglandular development.

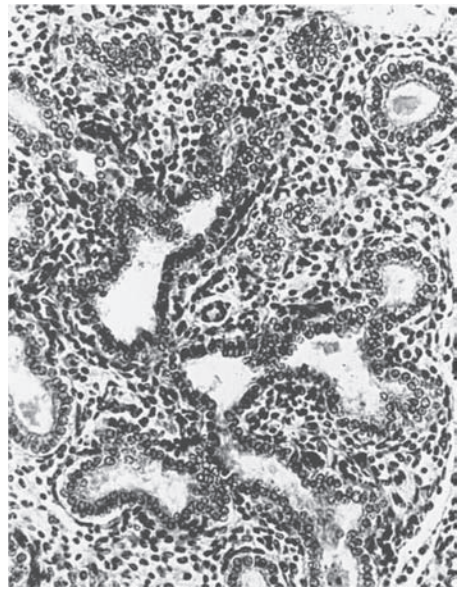


Figure 20.2. Early canalicular stage of development at 17 weeks' gestation. Vascularity is still poor and there is no blood–air barrier formation.

Canalicular Phase (17 to 27 Weeks)

Preacinar airways increase in diameter and, more peripherally, prospective respiratory bronchioles and then alveolar ducts begin to form. The mesenchymal tissues become more vascularized. The epithelial cells are characterized by abundant glycogen initially, but from about 20 weeks, and associated with differentiation, glycogen diminishes. Type 1 pneumocytes, stretched over capillaries, are identifiable by 22 weeks and type 2 cells shortly afterward (McDougall and Smith 1975) (Fig. 20.2). Around 23 to 24 weeks' gestation, capillaries are pushing into the airways, the first blood–air barriers are forming (about 0.6 μm) and there is sufficient area for respiration to occur (Fig. 20.3).

Alveolar Phase (28 Weeks to Term)

This phase is characterized by rapid maturation of the acinus. Airway growth prenatally is linear. At the end of the canalicular phase, the terminal acinus is more in the form of a saccule. Some alveoli start to develop between 28 and 32 weeks

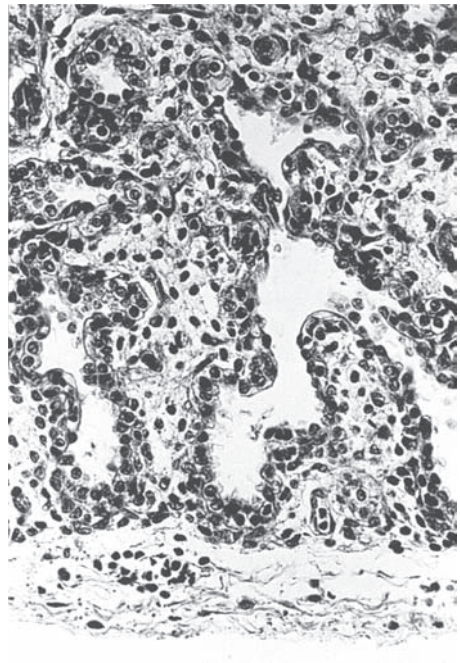


Figure 20.3. Canalicular stage lung at 23 weeks' gestation. Capillaries are starting to push into the airways, pulmonary epithelium is becoming attenuated and blood–air barriers are recognizable. Epithelium at distal sites, adjacent to the pleura remain cuboidal.

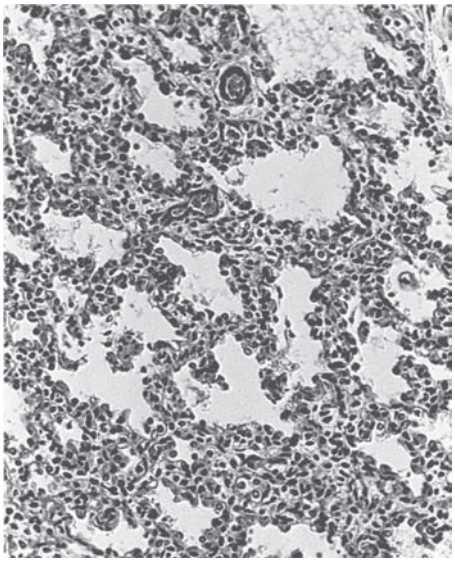


Figure 20.4. Terminal sac development at 28 weeks' gestation. Mesenchyme is less prominent and vascularization more marked.

but often retain a double capillary layer. Further septation leads to formation of true thin walled alveoli, and by 34 weeks mature cup-shaped alveoli line the elongated saccules. By term, between 30% and 50% of the adult number are present (Langston et al. 1984; Hislop et al. 1986; Hodson 1992). Alveolarization continues to about 8 years of age.

A characteristic of the late canalicular, early alveolar phase is the rapid differentiation of type 1 and type 2 pneumocytes (Fig. 20.4). The development of the former is reflected in the increasing numbers of blood–air barriers and surface area available for gaseous exchange, the latter in production of surfactant, a complex surface active secretion formed of phospholipid and protein. The lung interstitium diminishes, and by term there is little residual mesenchyme between respiratory units.

Lung Development and Control

Rapid progress has been made in recent years in this complex field. Normal lung development and function is dependent on a wide variety of factors, many of them extrinsic to the lung itself. Some of these factors are more conveniently discussed in the context of lung pathology, especially pulmonary hypoplasia.

The interaction between mesenchyme and the in-growing epithelial buds is fundamental. Indeed lung mesenchyme is a requirement for normal morphogenesis. The numerous factors involved in the complex interactions of short-range inducers in lung development have been subject to review (Shannon and Hyatt 2004; Groenman et al. 2005). The appearance and subsequent disappearance, during the transition from pseudoglandular to canalicular phase, of various laminins and integrin subunits in bronchial bud basement membranes imply a critical role at this stage of normal lung branching (Virtanen et al. 1996). Further, alterations in concentrations of different subunits around the time of type 1 and 2 pneumocyte appearance suggest a role in epithelial differentiation (Sigurdson et al. 1994; Durham and Snyder 1995, 1996). Growth factors, especially fibroblast growth factor 10, are critical from a very early stage possibly operating via sonic hedgehog, a regulatory molecule present in many organs and found especially at epithelial tips (Shannon and Hyatt 2004). Epidermal growth factor with receptor, and transforming growth factor-α, have been co-localized in airway epithelium in normal fetal lung (Strandjord et al. 1995). Insulin-like growth factor-2 has been localized in mesenchymal fibroblasts. Some of the mitogenic properties of the growth factors may be mediated via a local paracrine or autocrine action (Harding et al. 1993).

More systemic influences stem from hormones. Glucocorticoids, possibly acting synergistically with thyroid hormones, stimulate pulmonary fibroblasts to produce fibroblast-pneumocyte factor (FPF), which causes pulmonary epithelia to differentiate into type 2 pneumocytes, indicated by an increase capacity to produce surfactant (Smith and Post 1989). In vitro, this occurs rapidly (60 minutes), suggesting a posttranslational effect. Of interest, androgens appear to block FPF production, which might partly account for the increased risk of respiratory distress in male infants. Glucocorticoids may also stimulate the de novo synthesis of fatty acids, used by type 2 pneumocytes to produce surfactant (Rooney 1989). In contrast, thyrotropin-releasing hormone with or without dexamethasone depresses the late

gestation surge in antioxidants probably at the level of gene transcription rather than posttranscriptionally (Chen and Frank 1993).

Biochemical and Physiological Maturation

It is clear that the simple physical process of blood–air barrier formation that will permit rapid diffusion of gases to and from the pulmonary vasculature, and recognizable microscopically, is an important prerequisite for the transition to extrauterine life. However, successful transition is also dependent on the concurrent maturation of specific biochemical enzyme systems. While not readily assessable by the pathologist, an awareness of them can assist in the understanding of the problems of early neonatal life that produce respiratory difficulties, particularly in the preterm infant. Three main systems have been studied, although only a brief outline will be presented.

Surfactant

Synthesized by type 2 pneumocytes, surfactant is a compound formed mainly of phospholipid (80%; 50% of which is dipalmitoyl-phosphatidylcholine), cholesterol (10%), and at least four surfactant-associated proteins (SP-A to D) (10%).

Type 2 pneumocytes store surfactant in lamellar bodies, which are secreted onto the alveolar surface by exocytosis to form a monolayer of surface active material. Production and secretion appears to be further stimulated after birth by mechanical factors such as lung expansion (Wright and Clements 1987)). Once secreted, turnover is rapid, and approximately 10% to 30% is replaced per hour. The means by which surfactant is removed is not clear, but there is evidence that alveolar macrophages are involved and recycling occurs (Mendelson and Boggaram 1989).

Lung Liquid Secretion

The fetal lung is filled by a liquid, the production of which starts early in gestation and normally terminates only in the early stages of labor. Pulmonary epithelium actively secretes chloride ions into the duct lumen, and this passage of negative ions is accompanied by sodium and water (Olver and Strang 1974). At birth, lung liquid secretion needs to cease, and that already present needs to be absorbed. Adrenaline, to which pulmonary epithelium becomes increasingly sensitive as term approaches, appears to control this latter aspect (Brown et al. 1983). Its action on chloride transport is unclear, but adrenaline may open sodium channels and stimulate active sodium transport from the alveolar lumen (Walters and Ramsden 1987). Where there is a failure of this process, persistence of lung liquid may cause transient neonatal respiratory distress. Of greater importance to the pathologist is the evidence that indicates that the secretion of lung liquid and its gradual loss into the amniotic fluid is vital to normal lung growth (Nicolini et al. 1989).

Antioxidant Enzymes

Oxygen, even at normal inspired concentrations, is damaging to lung epithelium and endothelium because of the production of toxic radicals. Evolution has provided a number of defense mechanisms including some vitamins (A, C, and E), and enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Under normal circumstances, fetal lung is only exposed to low oxygen tensions, and consequently antioxidant enzyme levels are relatively low. In parallel with those of surfactant, antioxidant enzyme systems mature with gestation and reach a peak at term (Frank and Sosenko 1987). Recent evidence suggests that resistance/susceptibility to oxygen is determined less by baseline levels of antioxidant enzymes than by the response of those enzymes to hyperoxia. A deficient response has been detected in the prematurely born (Frank and Sosenko 1991).

Pulmonary Vascular Changes at Birth

Only some 10% to 12% of cardiac output passes through the pulmonary vasculature in utero, most bypassing it via the ductus arteriosus and foramen ovale. Intrapulmonary arteries are thick-walled, and endothelial cells are plump and overlap. At birth, partly as a result of increased oxygen

tension, pulmonary arteries dilate, vessel walls become thinner, and endothelial cells are stretched and appear flatter. In the precapillary arteries, the changes may occur within minutes after birth. Associated changes that promote further pulmonary blood flow include ductal closure. In healthy infants, most blood flow alterations have occurred by 8 to 12 hours after birth. Subsequently, in the first 4 days, more muscular arteries are "recruited" to the pulmonary circulation so that the cross-sectional area of the precapillary bed rises. This allows an increase in cardiac output through the pulmonary vasculature, but without a parallel rise in pulmonary vascular resistance. Over a period of weeks, these changes are "fixed" structurally, by smooth muscle hyperplasia, increases in the connective tissue of the media, and an increase in the internal elastic lamina (Haworth 1988).

Developmental Anomalies

Upper Respiratory Tract

Anterior and Posterior Nares

Total absence of the nose may result from failure of development of the nasal placodes or occur as part of a wider range of cerebrocranial abnormalities (Gifford et al. 1972). More often the nose is replaced by a blind-ending proboscis or lies superior to a single fused orbit. Many of these midline defects are associated with trisomy 13 (see Chapter 26 p. 706).

Attempts at passing a probe through the nares into the nasopharynx may reveal the presence of choanal atresia, which may be unilateral. It can be an isolated finding or be part of a wide range of anomalies including those of the ear, eye, cardiac defects such as Fallot's tetralogy, and cerebral abnormalities (*c*oloboma, *h*eart disease, *a*tresia choanae, *r*etarded *g*rowth, and *e*ar anomalies—the CHARGE association) (Pagon and Graham 1981).

Lips and Palate

Failure of the maxillary process to fuse with the nasal prominence gives rise to a lateral cleft, sometimes with alveolar margin involvement. Clefts of the lip and palate are common abnormalities seen both individually or together. In the absence of other malformations they are commoner in males, but the sex incidence is approximately equal in the presence of nonfacial malformation. There is a considerable list of potential associations and syndromes (Winter et al. 1988), and the underlying causes are extremely varied. Some midline clefts have a clear underlying genetic association such as with trisomy 13, but environmental agents such as smoking or drug ingestion may also be involved (Murray and Schutte 2004). In the Pierre–Robin sequence a displaced tongue interferes with normal palatal fusion.

Lower Respiratory Tract

Larynx

Laryngeal Atresia

This may occur at any level within the larynx, although there always appears to be some involvement of the supraglottic region. Smith and Bain (1965) classified nine examples into three types:

- *Type 1*: The vestibule is a shallow cleft flanked by the apices of the arytenoids. Below this is a mass of muscle with partially fused arytenoid cartilages, behind which is a fine pharyngotracheal duct, 1 mm in diameter. The cricoid is malformed and conical (Fig. 20.5).
- *Type 2*: The vestibule is normal and the arytenoid cartilages separate. The glottis is a blind cleft between the vocal folds, and the cricoid is dome-shaped with a pharyngotracheal duct passing posteriorly.
- *Type 3*: The glottis is occluded by a fibrous connective tissue membrane and a fused mass of lateral cricoarytenoid muscles. The vocal processes of the arytenoid are fused and the pharyngotracheal duct passes posteriorly.

Laryngeal atresia represents an arrest of normal development when there is failure of recanalization of the lumen during the 8th to 10th week of gestation, after it has been obliterated by proliferation of pharyngeal mesoderm (Zaw-Tun 1988). Although in the original description there was only one example of type 3, it is the more common and represents a late failure of recanalization.

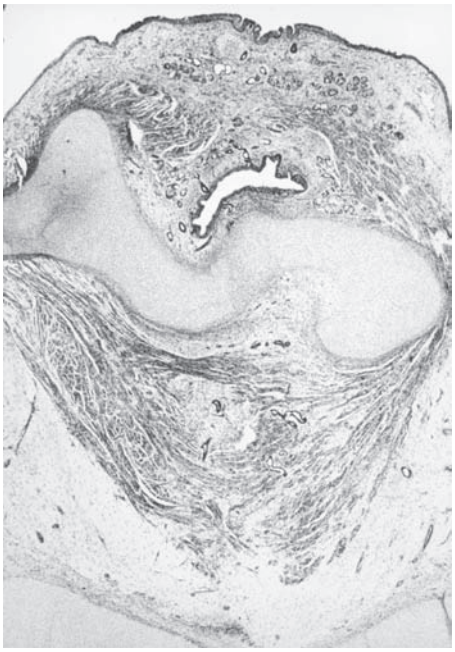


Figure 20.5. Transverse section across laryngeal atresia with abnormal, fused arytenoid cartilages. The pharyngotracheal duct passes dorsally and a mass of muscle is fused anterior to the cartilage.

Zaw-Tun (1988) regards the type 3 as having been described as a laryngeal web elsewhere and, as such, is less severe and often remediable; types 2 and 3 are rarely other than fatal (Hicks et al. 1996). Types 1 and 2 are more usually associated with malformation elsewhere, whereas type 3 is usually an isolated abnormality. Laryngeal atresia can be inherited as a component of Fraser's syndrome (Slovotinek and Tifft 2002)

In the severe forms of atresia the lungs may be hyperplastic because lung liquid efflux is obstructed. Indeed, the presence of lung hyperplasia is often the feature that alerts the pathologist to the presence of a laryngeal anomaly (see below).

Laryngeal Stenosis and Obstruction

Excluding laryngeal webs, which for pathogenetic reasons are discussed under atresia, most laryngeal stenoses are below the true vocal cords in the subglottis. Stenosis may be soft, due to fibrous tissue and mucous gland overgrowth, or hard (Fig. 20.6), due to cricoid cartilage overgrowth (McMillen and Duvall 1968), an abnormally shaped cricoid, or even a displaced first tracheal ring (Tucker et al. 1979). The laryngeal inlet may appear normal. A combined soft and hard tissue stenosis with posterior cleft has been described (Kaufmann and Kohler 1995). Although generally sporadic, a familial example of congenital subglottic stenosis has been described (Linna et al. 2004).

Laryngeal Clefts

Occasionally, clefts are anterior, but most laryngeal clefts are in the midline posteriorly and represent a failure of fusion of the tracheoesophageal septum. They have been classified as follows:

- Type 1: involving larynx only
- Type 2: partial cleft involving larynx and upper trachea
- Type 3: complete cleft involving trachea as far down as the carina (Lim et al. 1979)

Small clefts may be asymptomatic but neonates often suffer from stridor or respiratory distress (Fig. 20.7). For the more severe forms, mortality is high, and type 2 and 3 clefts require surgery for

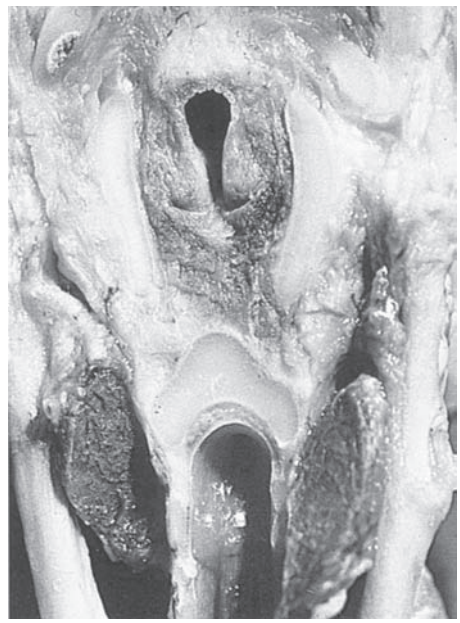


Figure 20.6. Laryngeal stenosis. Coronal slice through the larynx shows an abnormal bar of cartilage obstructing the lumen. (Courtesy of Dr. S. Knowles.)

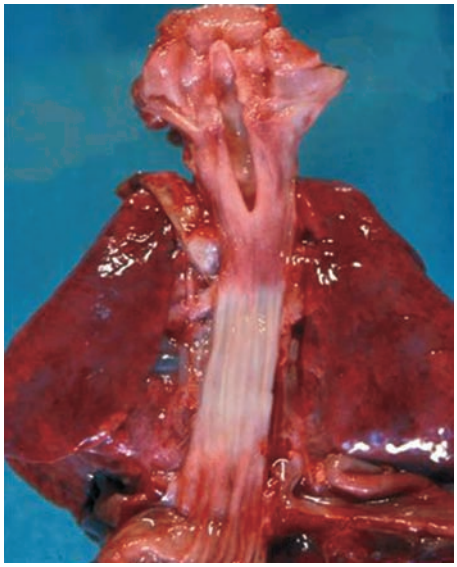


Figure 20.7. Laryngeal cleft (type 2) from a 34-week late neonatal death. (Courtesy of Dr. J. Keeling.)

survival (Samuel et al. 1997; Kubba et al. 2005). Up to 40% of type 1 clefts are associated with a tracheoesophageal fistula.

Laryngeal Cysts

Laryngeal cysts may present with neonatal stridor (90%) or respiratory distress (55%) usually on the first day of life (Mitchell et al. 1987). They may be endodermal in origin and have been classified as either saccular, when they are present in the laryngeal saccule, or ductal, when they result from distention of obstructed ducts (De Santo et al. 1970). Laryngoceles that develop in the saccule and contain air and fluid, may cause external compression (Chu et al. 1994). Obstruction by cystic hygroma and a hamartoma has been described (Thompson and Kasperbauer 1994; Fine et al. 1995). Those cysts with a mesodermal component, which may be partly extrinsic, may be more difficult to treat (Forte et al. 2004).

Laryngomalacia

A frequent clinical, though rare pathological, diagnosis, laryngomalacia is one of the commonest causes of congenital laryngeal, usually inspiratory, stridor (Friedman et al. 1990). Sometimes stated to be due to a soft cartilaginous framework of the larynx (Cotton and Richardson 1981), the pathology is very poorly defined and the histology may be normal or show little readily detectable abnormality (Manning et al. 2005). It has been suggested that stridor may result from a mild localized form of hypotonia due to neuromuscular dysfunction (Belmont and Grundfast 1984), although the neuromuscular problems may be more systemic (Chandra et al. 2001). Laryngomalacia resolves spontaneously during the second year in 90% of cases.

Trachea

There are about 22 cartilaginous rings between the lower border of the larynx and the carina. Most are simple C-shaped transverse rings, open posteriorly, but a significant number of half-rings or Y-shaped structures are present even in normal infants (Landing and Wells 1973). A reduction in ring numbers may be associated with Klippel–Feil syndrome and a wide variety of other conditions, including chromosomal disorders, skeletal dysplasias, and neural tube defects (Wells et al. 1990).

Tracheal Agenesis

Tracheal agenesis may be total or segmental, usually of the lower part, and is rare. It results from ventral or dorsal displacement of the tracheoesophageal septum. The agenetic segment of trachea may be long or short, and sometimes the agenetic segment is replaced by a thin fibrous cord. The distal trachea or bronchi may arise from the esophagus (Fig. 20.8) (Diaz et al. 1989), which can be a conduit for ventilation in some cases in the immediate neonatal period. However, prognosis is very poor (Lander et al. 2004). It is commonly associated with tracheoesophageal fistulas (Floyd et al. 1962; Bray and Lamb 1988) and other congenital abnormalities, particularly in the cardiac or genitourinary systems (McNie and Pryse-Davies 1970; Diaz et al. 1989).

Tracheal Stenosis

Tracheal stenosis may present as stridor or respiratory distress in the neonate or recurrent pneumonia in the older infant (Loeff et al. 1990). It may be due to an intrinsic abnormality of the trachea

or from extrinsic pressure. Intrinsic tracheal stenosis is either diffuse or segmental. Diffuse stenosis may result from a posterior fusion of the tracheal cartilages. In some cases the trachea is funnel shaped, and it gradually becomes more stenotic. Common associations include a sling left pulmonary artery or pulmonary agenesis. Diffuse stenosis may also be caused by a solid cartilaginous sleeve usually associated with craniosynostosis syndromes such as Apert's. It probably reflects a generalized mesenchymal defect. The most frequent stenosis is a segmental, napkin-ring type with individual cartilages having a complete tracheal ring.

More frequent than intrinsic tracheal stenosis, is extrinsic compression from a vascular ring encircling the trachea such as a double aortic arch, or an abnormal vessel such as an aberrant left pulmonary artery (Fig. 20.9). Many are associated with other abnormalities, notably of the cardiovascular system (Bonnard et al. 2003). About a third are diagnosed at birth, with a later presentation often being from dysphagia or nonspecific respiratory symptoms such as infection or respiratory distress.

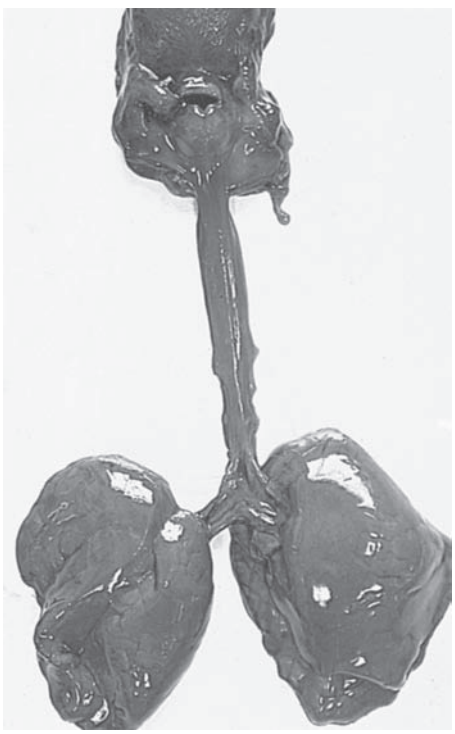


Figure 20.8. Tracheal agenesis in a 34-week gestation stillbirth. Laryngeal inlet appears relatively normal despite the absence of airway below the cricoid, and the bronchi are arising directly from the esophagus. Both lungs are of normal size.

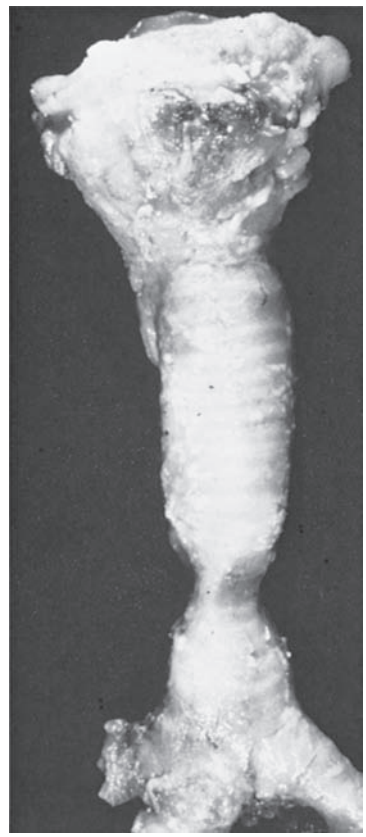


Figure 20.9. Localized tracheal stenosis in a baby with aortic arch anomalies and congenital heart disease.

Tracheoesophageal Fistula

A tracheoesophageal fistula is the most common congenital abnormality to affect the trachea, which almost invariably demonstrates abnormalities of the tracheal cartilages similar to those found in tracheomalacia. The usual variant of tracheoesophageal fistula is a blind-ending, upper esophageal pouch associated with a fistulous connection between the lower esophagus and the trachea (Holder and Wooler 1970). Tracheoesophageal fistula is discussed more fully in the section on the gastrointestinal tract (see Chapter 18 p. 469).

Tracheo- and Bronchomalacia

Tracheo- and bronchomalacia are due to an inadequacy of the respective cartilaginous frameworks and cause a loss of airway patency at some point in the respiratory cycle. They may present clinically as stridor or respiratory distress and diagnosis is made bronchoscopically by visualizing airway collapse, usually during expiration. As the problem can resolve spontaneously, and is more frequent in preterm infants, it has been attributed to "immaturity" of tracheal or bronchial cartilages (Cogbill et al. 1983), implying abnormally thin or floppy cartilage. A deficit in the cartilaginous rings may be present (Gupta et al. 1968; Belmont and Grundfast 1984; Benjamin 1984). Describing the normal cartilage to soft tissue ratio as 4.5:1, Benjamin states that the C-shaped cartilages in tracheomalacia are smaller and the ratio may fall to 2:1. The increase in the soft tissue posteriorly allows anteroposterior collapse of the airway. Familial malacia is reported (Agosti et al. 1974), and an association with other congenital abnormalities, particularly congenital heart disease, is common. There does appear to be a significant association between these tracheobronchial abnormalities and chromosomal anomalies such as trisomy 21 and 22q deletions (Maeda et al. 2000; Huang and Shapiro 2000; Betrand et al. 2003).

Secondary tracheomalacia, in this context implying acquired damage to the trachea, is a rare complication of chronically ventilated preterm infants. Damage to the cartilaginous framework is presumed to occur from recurrent tracheal infection or possibly trauma from the endotracheal tube (Sotomayor et al. 1986).

Lungs

Bronchial Abnormalities

Abnormalities of bronchial segmentation may be extra- or intrapulmonary. Many are incidental findings and of little significance, although some abnormalities of bronchial origin may present in later life with pneumonia. The commonest extrapulmonary anomaly is that of isomerism, in which bronchial development is similar on both sides (either left or right). These are associated with abnormalities of "left–right symmetry" elsewhere, especially of the heart (Landing 1984; Stewart et al. 1984; DeVine et al. 1991).

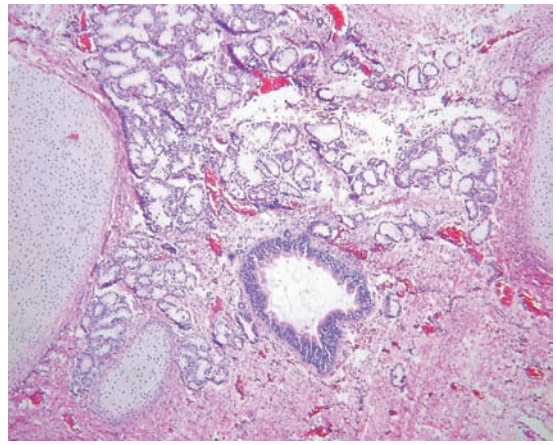


Figure 20.10. Atretic main bronchus in a 36-week-gestation baby. Lung distal to the atresia demonstrated solid (type 3) adenomatoid malformation.

Isolated bronchial atresia is generally considered extremely rare and usually apical. Some atresias have been associated with bronchogenic cysts, and unrecognized bronchial atresia may underlie the pathology of a number of pulmonary cystic diseases (Fig. 20.10) (see below). Although this has suggested a malformative basis for atresia (Kuhn and Kuhn 1992; Mori et al. 1993), the presence of fibrotic material in some cases suggest that at least a few are "acquired."

Bronchial stenosis is rarely caused by an intrinsic abnormality (Chang et al. 1968) but by extrinsic pressure. Enlargement and pressure from pulmonary arteries from pulmonary hypertension is the commonest association.

Pulmonary Agenesis

Bilateral pulmonary agenesis is extremely rare (Ostor et al. 1978) but unilateral agenesis is encountered occasionally (Booth and Berry 1967; Engellener et al. 1989; Cunningham and Mann 1997). Agenesis may be complete; associated with a rudimentary bronchus; or associated with a rudimentary bronchus with ill-developed pulmonary tissue. Unilateral agenesis may be associated with esophageal atresia and distal tracheoesophageal fistula. It has been suggested (Knowles et al. 1988) that unilateral pulmonary agenesis may

"replace" tracheoesophageal fistula as a component of the VACTERL association (vertebral defects, anal atresia, cardiac defects, tracheoesophageal atresia, renal anomalies, limb defects). The solitary lung may show the normal bronchial divisions, but a compensatory increase in alveolar number may produce mediastinal shift. Chromosomal abnormalities have not been recognized in association with pulmonary agenesis, although a recurrence has been reported (Podlech et al. 1995).

Other Pulmonary Lobar Anomalies

Herniation into the lungs into the neck has been described in iniencephalus and Klippel–Feil syndrome. A part of the right upper lobe may grow medial to the right posterior and common cardinal veins to form an azygos lobe. Fusion of the lung bases behind the pericardial sac forms a horseshoe lung. This is generally asymptomatic except when associated with other anomalies especially pulmonary vascular anomalies such as scimitar syndrome, in which there is right pulmonary venous drainage into the inferior vena cava (Figa et al. 1993).

Pulmonary Cystic Disease

A wide variety of pulmonary abnormalities can lead to a clinical diagnosis of a congenital pulmonary cyst or cysts. Presentation is also very variable, and while some may be symptomatic and present in the early neonatal period, a few may be discovered incidentally at autopsy. However, a significant proportion are now diagnosed in utero, often following routine anomaly ultrasound scan. At this early stage, there is a good argument for describing these lesions simply as cystic lung malformations (Bush 2001), as the precise diagnosis may need to await later pathological examination or other assessment. Indeed, although cystic lung disease is often described as a number of separate and seemingly distinct entities, there is often overlap.

Differential diagnosis typically includes adenomatoid malformation and sequestration with diaphragmatic hernia as the major nonpulmonary lesion. Cystic lung malformations can cause effusions or hydrops due largely to venous obstruction and mediastinal shift. Compression of adjacent normal lung may cause pulmonary hypoplasia (see below). Complications and management are more dependent on the size and extent of the lesion and factors such as the degree of mediastinal shift than precise subtype (Davenport et al. 2004). Increasing experience of antenatal detection indicates prognosis is not as bad as formerly thought, even in the presence of systemic complications. Pulmonary cysts may become less visible as gestation progresses and, by the time of birth, imaging may detect some lesions only with difficulty. Some argue that abnormal lung should always be resected, as it will almost invariably become symptomatic at some point in life (Laberge et al. 2005) and have a higher risk of malignancy (Ueda et al. 1977; Domitzio et al. 1990; Ribvet et al. 1995; Kaslovsky et al. 1997; MacSweeney et al. 2003). Others recommend postnatal follow-up with computed tomography (CT) scan for the first year, and resection only if the abnormality persists on imaging or is symptomatic (Calvert 2005). In this latter study 78% of infants diagnosed with cystic lung disease in utero required surgery in the their first year. It is likely that many cystic lung lesions go undetected and regress, although adult presentation is recorded (Lackner et al. 1996).

Bronchogenic Cysts

Bronchogenic cysts are usually an incidental finding in the perinate. Considered to result from an abnormal "late" budding of the primitive tracheobronchial tree, they may be intrapulmonary, anterior mediastinal, or occasionally apposed to the trachea in the neck. However, lesions with the histological characteristics of bronchogenic cysts have been described in a very wide range of locations, such as infradiaphragmatically or in subcutaneous tissues, often with little obvious connection with the respiratory tract. Bronchogenic cysts should not be in communication with tracheobronchial tree. The epithelium is ciliated columnar, but squamous metaplasia can be present. Although some searching may be necessary, bronchial glands or cartilage in the cyst wall is the most reliable histological means of distinguishing bronchogenic from esophageal cysts (Salyer et al. 1977).

Congenital Lobar Emphysema

This usually presents in the neonatal period or sometimes slightly later with respiratory distress due to lobar overexpansion and compression of adjacent tissues. In one study, approximately 50% presented in the first week of life and 80% by 6 months. It almost invariably affects the upper lobes, but multiple lobes can be involved (Mani et al. 2004). The key factor in congenital lobar emphysema (CLE) is largely related to partial obstruction of the airway and air trapping. Usually, the abnormality is intrinsic to the bronchus and bronchial stenosis, possibly due to a cartilage defect, as has been reported (Warner et al. 1982), but extrinsic bronchial compression with CLE has also been described (Engle et al. 1984). Histopathological findings are often limited. The affected lung shows overexpanded alveoli but the underlying structure is usually normal. Identifying the cause of the obstruction with confidence may be impossible.

Congenital lobar emphysema has been diagnosed in utero, and the overexpansion of the lung in this context is probably due to impaired lung liquid flow by bronchial obstruction. The alveoli may be simplified, large, and show increased elastic in the alveolar walls.

Congenital Pulmonary Adenomatoid Malformation

Although still widely described as congenital cystic adenomatoid malformation (CCAM), the general term *congenital pulmonary adenomatoid malformation* (CPAM) acknowledges that cystic change may not be a feature of some malformations. Adenomatoid malformation has been widely classified by the system (types 1 to 3) devised by Stocker et al. (1978) from pediatric lung resections to which two further subtypes (types 0 and 4) have been added more recently. However, it can be difficult to classify some adenomatoid malformations using this system, especially lesions seen in fetal life (Cha et al. 1997) or resected shortly after birth. Overlap with other lesions (Bale 1979; Fisher et al. 1982) occurs. While aspects of the pathogenesis remain incomplete, Langston (2003) places more emphasis on regarding some pulmonary cysts as part of a sequence that makes it easier for some lesions, which often have more than one pathology, to be more easily

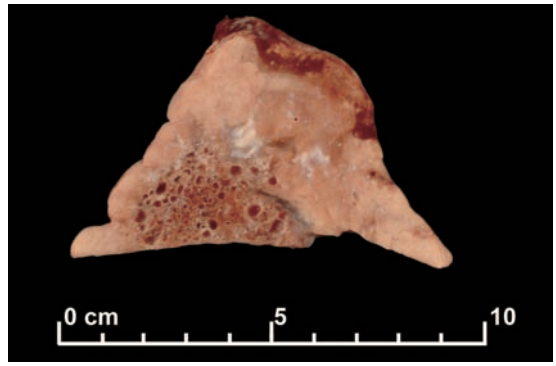


Figure 20.11. Small cyst adenomatoid malformation (type 2) in part of a left lower lobe.

described and understood (Fig. 20.11). Bronchial atresia may be a common factor in a number of types of pulmonary cystic disease (Riedlingen et al. 2006). Classification is as follows:

- *Large cyst type (Stocker type 1):* Often affecting only a single lobe, these cysts may be up to 3 to 7 cm in some children. Even in small babies the cysts may be 2 cm in diameter, with smaller cysts merging with adjacent lung. The cysts can cause mediastinal shift or even hypoplasia of adjacent lung. These cysts communicate with the bronchial tree. Microscopically, cysts are lined by respiratory-type ciliated columnar epithelium, which may be flattened, or a mucigenic epithelium (Fig. 20.12). The wall comprises a thin fibromuscular layer, the cysts lie back to back, and small islands of cartilage may be present. Cysts with the appearance of enlarged alveoli may be interspersed between the larger cysts.
- *Small cyst type (Stocker type 2)*: Small cyst type adenomatoid malformation presents with a wide spectrum of appearance. Multiple, evenly spaced cysts may rarely occupy a whole lung but, more usually, only a lobe or part of a lobe. Small cystic disease may even be scattered throughout a lobe of lung. The epithelial lining and wall of the cysts usually resembles that of normal respiratory bronchioles (Fig. 20.13) and, on occasions may be difficult to distinguish from normal structures except that they are present to excess. Cartilage is not present. Detailed study suggests bronchial anomalies, especially atresia (Imai and Mark 2002), may be

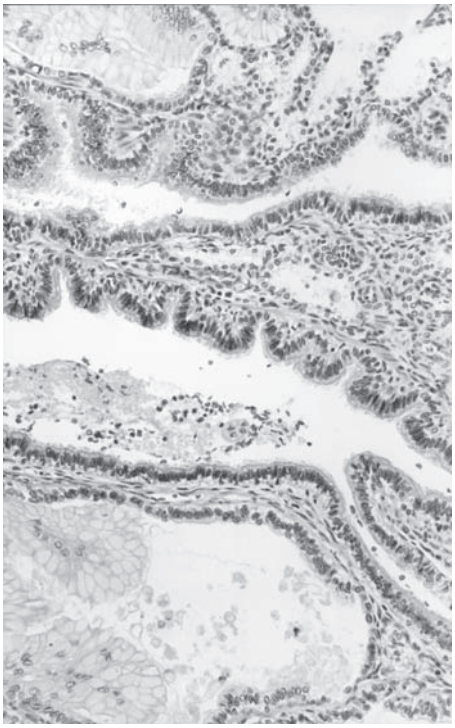


Figure 20.12. Congenital cystic adenomatoid malformation (CCAM) type 1 showing typical epithelium with thin layer of fibromuscular tissue. Metaplastic, mucous epithelium is characteristic of type 1 but is not always found.

present, and indeed Langston (2003) considers that this abnormality is usually, if not always, secondary to early airway obstruction. Mucus and macrophages, within the cysts and surrounding lung, suggesting airway obstruction, are common features of resected lung with small cyst disease. Very occasionally, striated muscle may be found randomly distributed throughout the malformation (Fig. 20.14). Of the three main subtypes, this type is most commonly associated with malformation elsewhere, especially of the renal tract (Pham et al. 2004).

- *Solid adenomatoid malformation (Stocker type 3)*: This subtype is formed of small cysts less than 0.5 cm in diameter, involving an entire lobe. Cyst lining is cuboidal epithelium on a very thin fibromuscular layer with no cartilage or mucigenic cells (Fig. 20.15). Due to the overall size of the affected lung, type 3 CCAM is more likely to produce significant mediastinal shift and carry a poor prognosis. This form of abnormality may have parallels with pulmonary hyperplasia due to airway obstruction (Langston 2003), and bronchial atresia has occasionally been demonstrated (Fig 20.10).
- *Peripheral cyst type (Stocker type 4)*: Proposed in 1994 (Stocker 1994), this type was considered a probable hamartomatous malformation of the

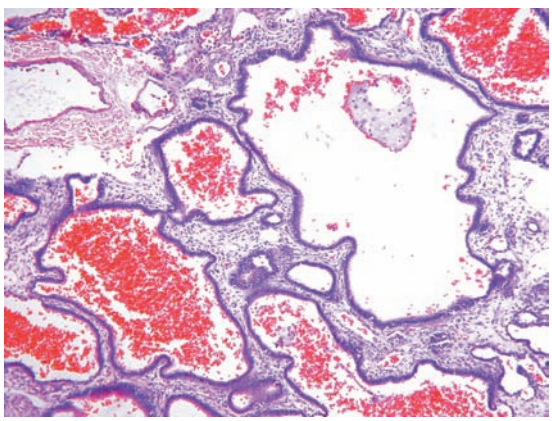


Figure 20.13. Small cyst adenomatoid malformation. Cysts are bronchiolar in appearance. This was an unexpected finding in a fetus terminated for renal agenesis.

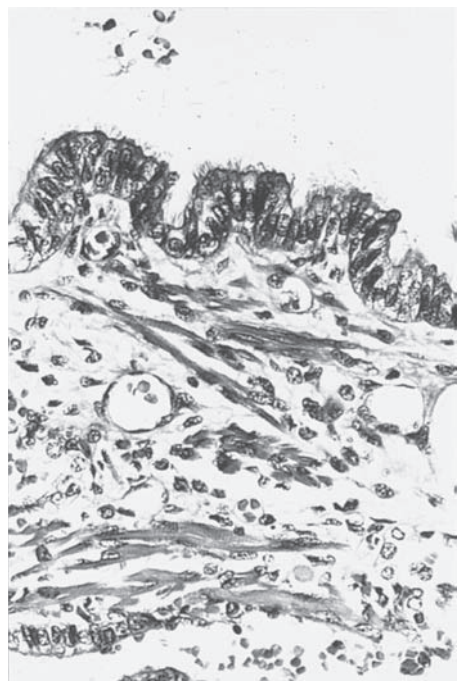


Figure 20.14. Small cyst adenomatoid malformation with striated muscle cells in the wall. The striations are visible.

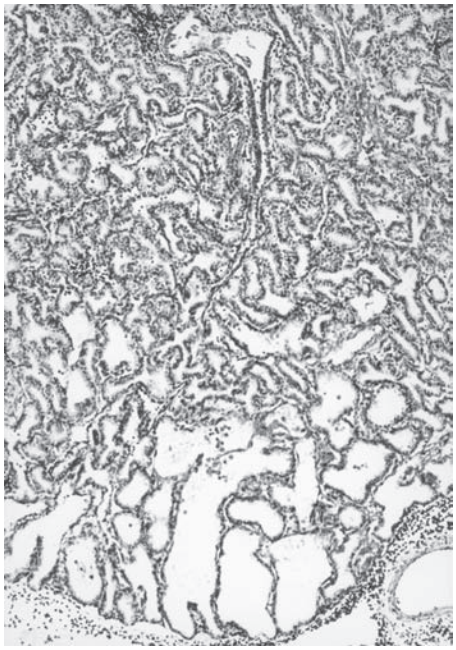


Figure 20.15. Solid, type 3 CCAM with uniform small cysts lined by cuboidal epithelium, which is surrounded by a very thin connective tissue layer.

distal acinus, often presenting as an asymptomatic incidental finding or sudden respiratory distress from spontaneous pneumothorax. It comprises large peripheral thin-walled cysts up to 7 cm in diameter lined by type 1 alveolar and cuboidal cells. The cyst walls are formed of thin loose mesenchyme often with thick-walled arteries. However, it is likely many of these cysts are cystic pleuropulmonary blastomas, and the distinction may be extremely difficult (MacSweeney et al. 2003). Indeed, because of the malignant potential of the latter, the diagnosis of a malformation should be made only with extreme caution (Hill et al. 2004; Miniati et al. 2006).

- *Acinar dysplasia (Stocker type 0)*: Initially reported as acinar dysplasia (Rutledge and Jensen 1986), this type has been proposed as a type 0 adenomatoid malformation. Incompatible with life, the macroscopic appearance is of a severely hypoplastic lung (Chambers 1991). It is described in association with cardiac anomalies and, in one case, renal anomalies (Gillespie et al. 2004). Microscopically, acinar development is extremely poor with terminal sacs lined by pseudostratified, bronchial-type columnar epithelium with goblet cells (Fig. 20.16).

Pulmonary Sequestration

Sequestered lobes are abnormal masses of pulmonary tissue that do not communicate with the main pulmonary bronchial tree and are supplied by an anomalous artery usually arising from the descending aorta. Sequestration may be intrapulmonary (intralobar sequestration, ILS), or extrapulmonary (extralobar sequestration, ELS), when the sequestered lobe is invested by its own pleura. Extralobar sequestration may present in the fetus with hydrops, as an incidental finding by ultrasound, or postmortem associated with other anomalies. In infancy it may present with respiratory distress. Extralobar sequestration may lie anywhere in the thorax or even subdiaphragmatically. In ELS, small cyst type adenomatoid malformation is commonly present.

Intralobar sequestration is usually found within the left lower lobe. It has been suggested that, because ILS is rarely found in fetuses or neonates (Ng et al. 1994) and presentation is often late, only

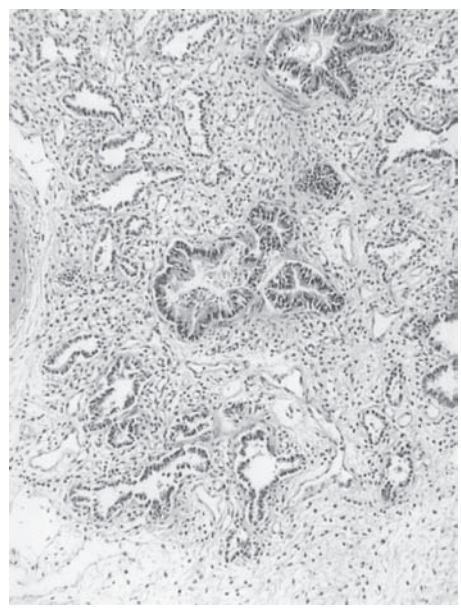
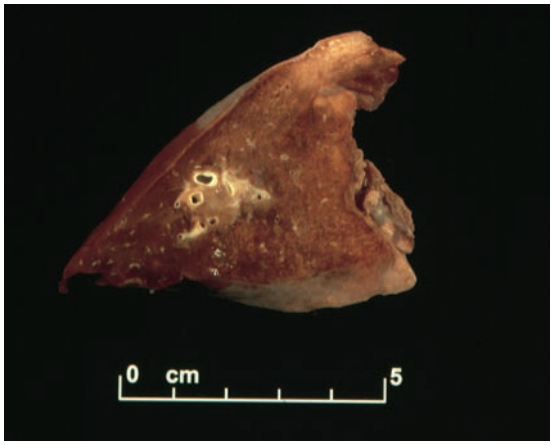
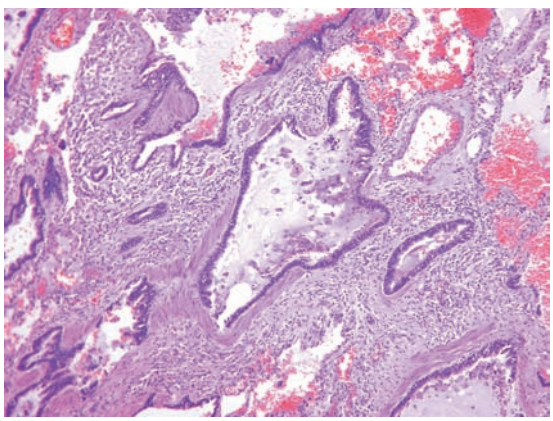


Figure 20.16. Acinar dysplasia from a term infant dying at 1 day of age. The central bronchiole is essentially normal, but distal to it there is no normal alveolar development. (Courtesy of Dr. C.J.H. Padfield, Nottingham, England.)



A



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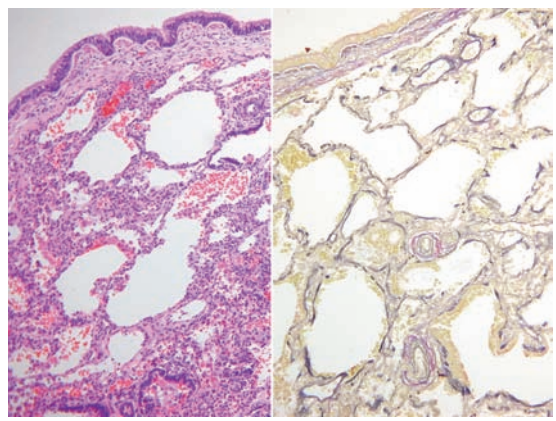

C

Figure 20.17. (A) Cystic lung diagnosed in utero (as CCAM). Resection of intralobar sequestration (ILS) with systemic vessel artery from the aorta. Sequestered lung is ill defined macroscopically, although the abnormal vessel plexus seen here shortly after it enters the lung. (B) Small cyst adenomatoid malformation in ILS with secondary changes of fibrosis and chronic inflammation in surrounding lung. Mucus retention and cellular debris within cysts is present. (C) Lung within ILS may not be overtly abnormal, but the special stain clearly shows elastic completely surrounding the alveoli. Elastic stain can often delineate the abnormal lung from normal quite sharply.

a few may be true malformations, and most are the result of infection (Stocker and Kagan-Hallet 1979). However, it is clear that a number of cystic pulmonary lesions, initially seen in utero by ultrasound, when resected postnatally are found to be supplied by a systemic artery (Walford et al. 2003) and conform to the definition of a sequestration (Fig. 20.17A) (Langston 2003; Orpen et al. 2003). In resected lung, small cysts are sometimes macroscopically visible in the resected specimen, but in others the adenomatoid component is only apparent histologically (Fig. 20.17B); mucus retention may be striking. The intervening lung parenchyma may also be abnormal; elastin stains may be very helpful in defining the extent of the abnormality (Fig. 20.17C).

Clements and Warner (1987) emphasize that a wide range of bronchial connection, arterial supply, and venous drainage may be found. The embryological origin of pulmonary sequestration is a matter of debate, although there does appear to be a consensus that other bronchopulmonary abnormalities may be related (Heithoff et al. 1976; Landing and Dixon 1979; Clements and Warner 1987).

Sequestered lung that communicates with the gut, most commonly the esophagus or stomach, is generally termed a bronchopulmonary foregut malformation. It is generally right sided and presents in infancy either as an associated finding with other malformations or as a symptom related to infection (Srikanth et al. 1992).

Pulmonary Heterotopias/Hamartomas

Solid hamartomas are rare anomalies and may contain cartilage and adrenal (Bozic 1969); pancreatic heterotopia has also been described

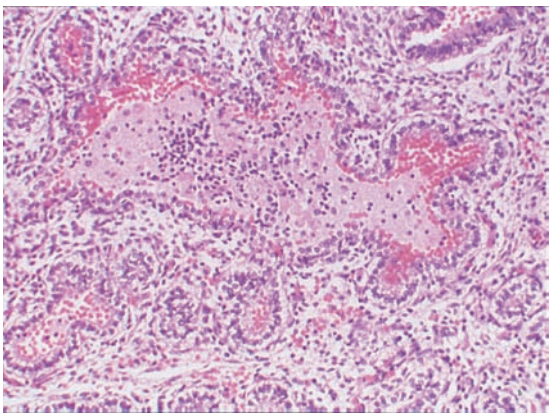

Figure 20.18. Heterotopic neural tissue in the airway of anencephalic fetus. This heterotopic material is probably aspirated.

(de Krijger et al. 2004). Glial and skeletal muscle masses are sometimes seen in anencephaly (Fig. 20.18) (Morgan et al. 2003). Rhabdomyomatosis or diffuse heteroplasia of skeletal muscle is described and is usually associated with a cardiovascular malformation (Chi and Shong 1982; Chellam 1988, Chen et al. 1991; Hardisson et al. 1997).

Alveolar Capillary Dysplasia

This dysplasia results from failure of normal alveolar capillarization, in which capillaries fail to push into the developing alveolar walls. The lungs are not hypoplastic but in some respects maturation appears arrested because of both the poor vascularization and the relative immaturity of the epithelium (Fig. 20.19A). It is associated with an

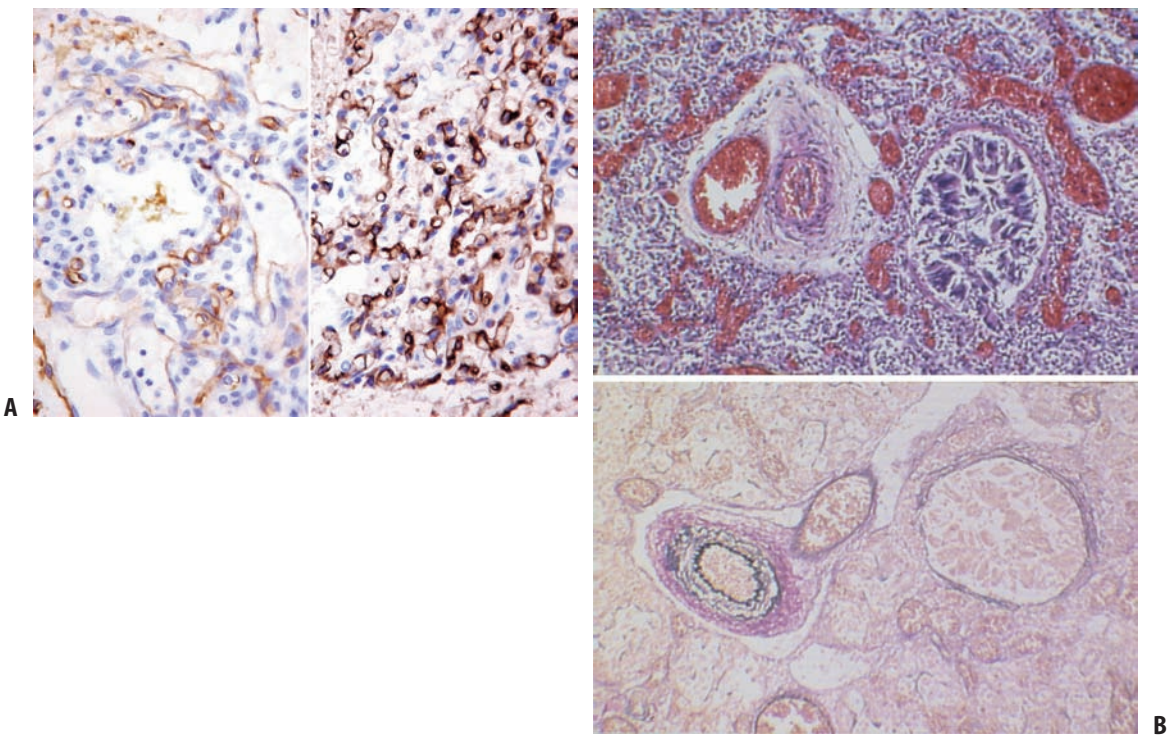

Figure 20.19. (A) Vascular pattern in alveolar capillary dysplasia (left) compared with normal lung at 33 weeks' gestation. CD-34 immunostain is used to highlight the capillaries. The capillaries in alveolar capillary dysplasia (ACD) are reduced in number and do not show the normal intimate relationship with the alveoli. The alveolar epithelium is immature for gestation. (B) Misalignment of the pulmonary veins in a term baby dying at 14 days with severe pulmonary hypertension of uncertain etiology. Highlighted in the elastic van Gieson (EVG) stain, the pulmonary vein accompanies the pulmonary artery adjacent to a terminal bronchiole. Although congested, dilated capillaries are present within the interstitium. There is an absence or severe reduction in capillaries pushing into the alveolar wall.

abnormality of the vascular bundle, sometimes known as misalignment of the pulmonary veins, such that there is both pulmonary artery and vein in the same adventitial coat (Fig. 20.19B). Babies present with severe ventilatory difficulties and marked pulmonary hypertension (Janney et al. 1981; Wagenvoort 1986; Cater et al. 1989; Langston 1991; Oldenburg et al. 1995; Guttierez et al. 2000). An association with other malformations is being increasingly recognized, and there is evidence that some case are autosomal recessively inherited (Sen et al. 2004).

Lymphangiectasia

Lymphangiectasia, or cystic dilatation of pulmonary lymphatics, may be primary or secondary. The former is rare and probably results from failure of pulmonary lymphatics to establish connections with the thoracic duct (Laurence 1955; France and Brown 1971). Secondary lymphangiectasia is usually associated with cardiac malformations, particularly anomalous pulmonary venous drainage (Esterly and Oppenheimer 1970). In contrast to interstitial emphysema, which it grossly resembles and in which air is trapped in the interstitial tissues, the lungs are firm, inelastic, and heavy. Bilateral pleural effusions are usual and these may be chylous. Histologically, dilated lymphatic spaces are present within interlobular septa, beneath the pleura, and between large vessels at the hilus of the lung.

Lung Hypoplasia

Although lung hypoplasia is a congenital condition, it is not a malformation, and is almost invariably secondary to pathology outside the respiratory tract. It is considered here at some length, first, because it is one of the most common abnormalities encountered in perinatal pathology; second, because of its varied pathogenesis; and third, because of the impact the study of lung hypoplasia has had on the study of normal lung growth.

Lung hypoplasia is common and has been estimated to occur in up to 14% of perinatal autopsies (Wigglesworth and Desai 1982; Husain and Hessel 1993). In stillbirths, it may be an incidental finding. In neonates, however, it can present within minutes or hours of birth and simulate intractable asphyxia, so it is an important pathological diagnosis (Devlieger et al. 1994). Occasionally, a clinical diagnosis of lung hypoplasia is not associated with immediate death, and postnatal changes in the lung may make pathological confirmation of the diagnosis difficult or impossible.

In most instances, however, diagnosis is straightforward, with macroscopically small lungs in a small thoracic cage (Fig. 20.20). After removal as a block, the diaphragmatic surfaces of the lungs are not in line with the apex of the heart (Fig. 20.21). Another readily available measure is lung/body-weight ratio; a lung/body-weight ratio of 0.015 before 28 weeks' gestation, and one of 0.012 at 28 weeks' gestation or later is indicative of hypoplasia. The lungs should always be greater than 1.2% of body weight (Askenazi and Perlman 1979; Wigglesworth and Desai 1981). However, ratios need to be interpreted cautiously in the presence of pathology such as infection or significant postnatal survival.

Histomorphometrically, hypoplastic lungs demonstrate reduced radial alveolar counts (Emery and Mithal 1960), although this may be a difficult assessment as a "one-off" procedure, and normal standards may vary (Cooney and Thurlbeck 1982).

Two broad patterns of histology in lung hypoplasia have been described (Wigglesworth et al. 1981), although these are not always sharply

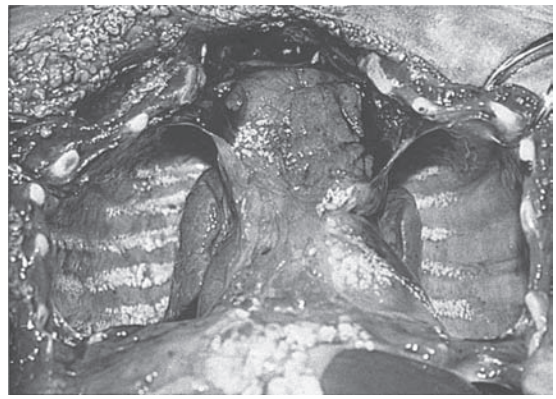

Figure 20.20. Thoracic contents of a 33-week early neonatal death with idiopathic fetal hydrops. The pleural effusions have been removed to show marked bilateral pulmonary hypoplasia.

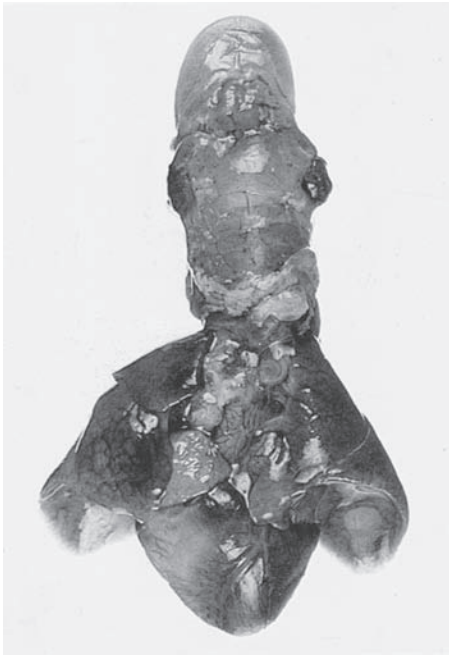

Figure 20.21. Hypoplastic lungs in relation to the size of the heart. Normally, the inferior surfaces of the lungs and apex of the heart should all be at approximately the same level. (From an early neonatal death at 26 weeks of gestation with renal agenesis.)

delineated (Nakamura et al. 1992). In the first pattern, the lungs appear immature as well as poorly grown. This manifests itself as narrow airways, retardation of epithelial differentiation, and delay in development of blood–air barriers. Evidence suggests that this is due to failure of differentiation of undifferentiated cells into type 1 pneumocytes. In the second pattern, the lungs are poorly grown, but maturation is appropriate for the gestation of the infant.

The poor maturation with poor growth pattern is especially associated with oligohydramnios-related hypoplasia. However, this simple division at the structural level may not always reflect events at the biochemical or functional level. For instance, the percentage of type 2 pneumocytes is similar in oligohydramnios-associated hypoplastic lungs compared with normal controls. Evidence of deficient surfactant production in the former suggests that there may be functional impairment of this cell type (Haidar et al. 1991). Functional impairment of type 2 pneumocytes may also occur in the hypoplastic lungs associated with congenital diaphragmatic hernia, particularly in the ipsilateral lung, and surfactant deficiency may contribute significantly to the functional impairment (Wilcox et al. 1997).

Mechanisms and Causes of Lung Hypoplasia

At first glance, the many associations and causes of lung hypoplasia have little in common, but the study of these seemingly disparate pathologies has contributed significantly to our understanding of normal lung growth (Wigglesworth 1987a,b). Pulmonary hypoplasia can be classified in a logical manner (Table 20.1) and based on factors that may impair normal growth. Some influences may be relatively subtle but operate early in development

Table 20.1. Mechanisms and causes of lung hypoplasia

Reduction in thoracic volume	Skeletal dysplasias	Thanatophoric dysplasia Achondrogenesis Asphyxiating thoracic dysplasia
	Pleural space lesions	Diaphragmatic hernia (often unilateral hypoplasia) Eventration Pleural effusions, e.g., in hydrops
Impairment of fetal breathing	CNS damage	Anencephaly involving brainstem Hypoxic-ischemic injury
	Congenital muscular disease	Congenital muscular dystrophy
Oligohydramnios	Reduced production	Renal agenesis Renal cystic dysplasia Lower urinary tract obstruction
	Increased loss	Prolonged rupture of membranes
Primary/other	Idiopathic Cytogenetic Familial Growth restriction	Trisomy

(Maritz et al. 2005), and the following underlying molecular mechanisms are only beginning to be understood (Groenman et al. 2005):

- *Adequacy of thoracic volume:* Not surprisingly, for lung to grow normally, it must have sufficient space in which to do so. Reduction in thoracic volume can be caused by poor rib growth, for example, skeletal dysplasias; a space-occupying lesion, for example, an abnormal viscus associated with diaphragmatic hernia (Areechon and Reid 1963); eventration (Fig. 20.22); or pleural effusion associated with hydrops. With hernias and eventration, the hypoplasia is typically unilateral.
- *Fetal breathing*: In utero, the fetus normally makes bursts of rapid but low-amplitude breathing movements, primarily diaphragmatic in origin. It is not entirely clear how or why such movements should be important to lung growth, but that they are is strongly suggested by lung hypoplasia in conditions associated with their absence, or by experimental work in which the effects of fetal breathing are negated (Liggins et al. 1981). It has been suggested that these movements allow influx of amniotic fluid or generate important pressure changes within the thorax (Wigglesworth 1987a; Kitterman 1984). The interrelationship among normal fetal breathing movements, lung liquid, and lung growth has been summarized (Hooper and Harding 1995). Pulmonary hypoplasia may also be seen when fetal breathing has been inhibited by severe brainstem injury (Endo et al. 2001) or other cerebral pathology (Matturri et al. 2003).
- *Oligohydramnios*: Oligohydramnios due to either insufficient production or excessive loss of fluid (Hislop et al. 1979; Nimrod et al. 1984), is probably the commonest recognized single cause of lung hypoplasia. In the presence of premature membrane rupture, pulmonary hypoplasia is one of the main determinants of survival, especially in the very premature (Robson et al. 1993; Lauria et al. 1995). The mechanism is unclear. In oligohydramnios, a larger than normal pressure gradient between fetal lung and amniotic sac occurs and the efflux of fetal lung liquid may be too rapid (Nicolini et al. 1989; Harding et al. 1990; Kizilcan et al. 1995). Lung liquid, retained within fetal airways and developing respiratory units, might act as a "stent" around which alveoli form. Another factor may be increased spinal flexion from uterine compression (Albuquerque et al. 2002). These hypotheses are not mutually exclusive.

The relevance of intraalveolar pressure can be seen in a rare combination of abnormalities sometimes found in Fraser's syndrome—laryngeal atresia or stenosis and renal agenesis. Despite the oligohydramnios due to renal agenesis, which would be expected to cause lung hypoplasia, the lungs are not hypoplastic but are either of normal size or even hyperplastic (Fig. 20.23). The laryngeal anomaly prevents the loss of fetal lung liquid (Wigglesworth et al. 1987; Silver et al. 1988). However, attempts to reproduce this experimentally by plugging the trachea give mixed results. While lungs are larger, lung maturation remains impaired (Piedboeuf et al. 1997; Nardo et al. 1998; Chapin et al. 2005). In human fetuses, there are also indications that tracheal occlusion does not enhance maturation, but the increase in lung size is due mainly to emphysema and mucus pooling (Heerema et al. 2003).

It is rare for a specific cause of lung hypoplasia not to emerge from careful study, although instances of "primary" pulmonary hypoplasia are recorded (Swischuk et al. 1979). Before a diagnosis of idiopathic or primary lung hypoplasia is entertained, some aspects of a case may be worth reassessing. In particular, the face (Potter's facies) or placenta (amnion nodosum) should be examined for evidence of oligohydramnios and radiographs for possible skeletal dysplasia. Pathology or malformation involving the brainstem should also be specifically sought. Primary muscle disease may also be very easily overlooked (Devlieger et al. 1994).

In some instances there is evidence that there is a genetic basis for the poor lung growth; hypoplasia has been associated with chromosomal abnormality such as trisomy 21 and 18 (Page and Stocker 1982), recorded in families and sets of twins.

Acquired Pathology

Broadly, the acquired pathology of the respiratory tract can be related to pulmonary

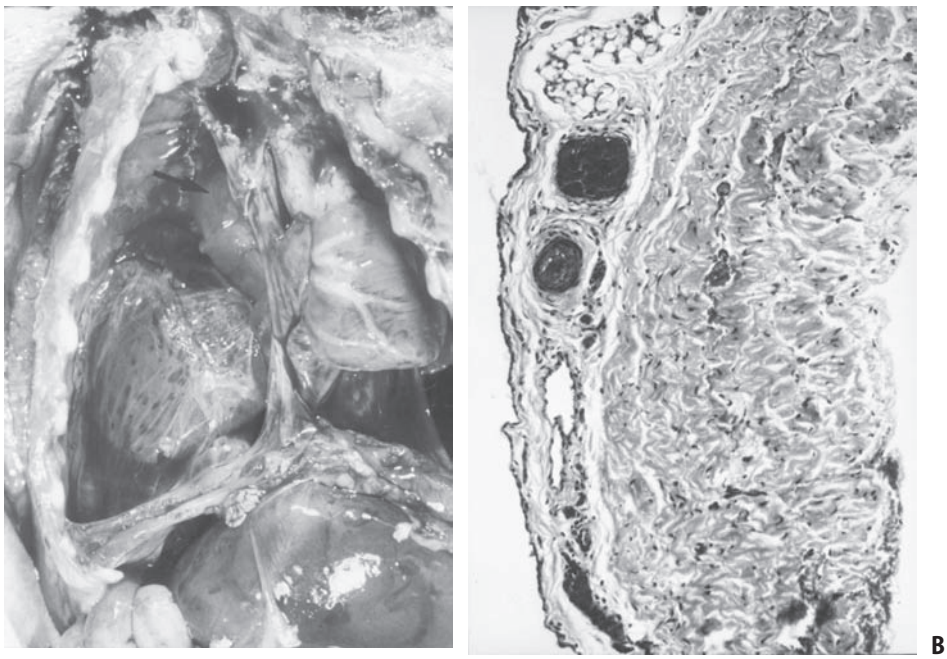

Figure 20.22. (A) Eventration. Right hemidiaphragm is a thin glistening membrane through which the right lobe of the liver can be seen. There is mediastinal shift, and the right lung is grossly hypoplastic (arrow). In this case, the left lung was also hypoplastic (not visible). From a term infant dying at a few hours of age. (B) The membrane comprises fibrous diaphragmatic tissue only with no muscle.

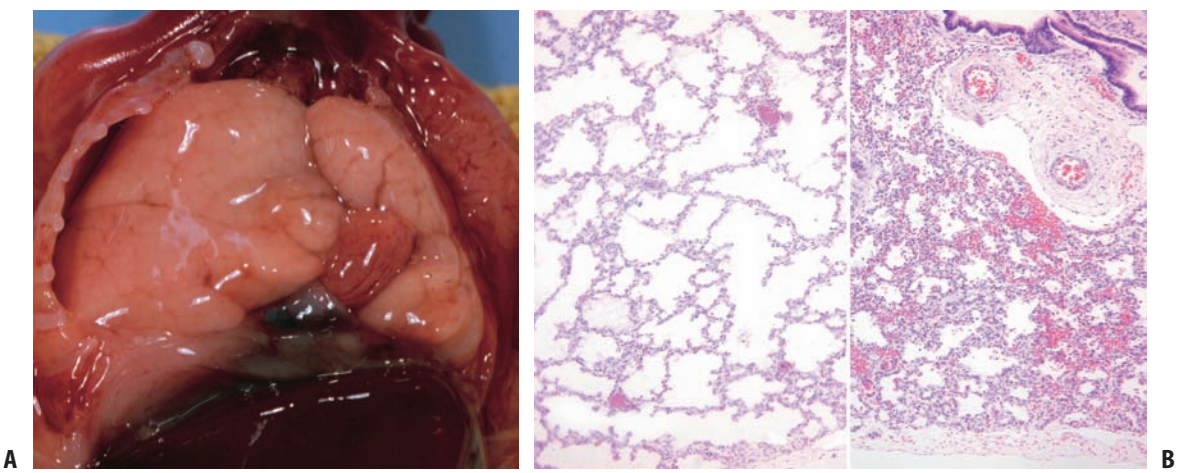

Figure 20.23. (A) Hyperplastic lungs at 19 weeks' gestation caused by laryngeal atresia. The bulky lungs cover the heart and the diaphragm is flattened. (B) Hyperplastic lung at 33 weeks' gestation (left) from a stillborn baby with laryngeal atresia compared with lung of similar gestation baby (right; same magnification). The hyperplastic lung appears inflated and more mature than might be expected.

immaturity, the consequences of birth asphyxia, or infection. Superimposed on this can be the effects of therapy, especially ventilation. Where the underlying pathology of lung and pathology of therapy are inextricably combined, it will be described here. A more detailed discussion of underlying mechanisms and pathology such as the direct effects of intubation are considered elsewhere (see Chapter 17).

Pathology of Immaturity

As discussed above, there are two aspects of pulmonary immaturity that together lead to respiratory problems: physical immaturity with inadequate surface area for efficient gaseous exchange, and, equally important, biochemical immaturity. Surfactant production may be inadequate, and a lack of antioxidant defenses may increase susceptibility to injury.

The lungs of most immature infants who have survived more than a few hours demonstrate pathological changes, but occasionally they are histologically normal. This is usually confined to the extremely preterm infant of around 23 to 25 weeks' gestation, in whom death has occurred within minutes or hours of birth. Death may be attributable to pulmonary immaturity alone.

Respiratory Distress Syndrome

The terms *respiratory distress syndrome* (RDS) and *hyaline membrane disease* (HMD) are frequently used interchangeably. However, for clarity, RDS should be considered a clinical term describing an acute illness, generally developing within 4 to 6 hours of birth in a preterm infant. There is a constellation of symptoms: increased respiratory rate (>60 breaths/min), respiratory distress (sternal and subcostal recession), cyanosis, and grunting that does not resolve within 24 hours. Radiographically there may be pulmonary collapse and an air bronchogram. Approximately 1% of babies develop RDS, the risk of which is inversely proportional to gestational age, with some 50% developing the syndrome before 30 weeks' gestation. The incidence and outcome has been radically altered by the use of prenatal steroids and replacement surfactant.

The majority of cases (75–80%) are associated with hyaline membrane disease, but, among others, respiratory distress can be caused by infection, birth asphyxia, massive pulmonary hemorrhage, and cerebral intraventricular hemorrhage (Wigglesworth 1977). It is possible that some babies die from other major problems before hyaline membranes develop.

Hyaline Membrane Disease

Hyaline membrane disease is a pathological term describing the presence of eosinophilic amorphous material lining the terminal airways of the neonate. It is usually seen in the lungs of very preterm infants (Farrell and Avery 1975), and most commonly is associated with prematurity and surfactant deficiency. However, it can also be associated with severe acute asphyxia, some forms of pulmonary infection, and pulmonary hemorrhage. It is almost invariably accompanied by respiratory distress, although the clinical syndrome may be obscured if the infant is ventilated, and some extremely preterm infants (<26 weeks' gestation) may become apneic rather than develop classic RDS. The natural clinical course has been further modified by the use of replacement surfactant, which may be used prophylactically very shortly after birth or as later rescue therapy (Halliday 2003).

Hyaline membrane disease associated with surfactant deficiency, and not treated by replacement therapy, usually presents an hour or so after birth with respiratory distress. With the exception of the most immature infants, the majority of infants survive with appropriate ventilatory therapy, unless additional pathology such as intraventricular hemorrhage supervenes. Ventilation is often required for a few days, but toward the end of the first week, and presumably reflecting a resurgence in surfactant levels, ventilatory requirements decrease.

Should infants die within a few hours of onset of the disease, the lungs are collapsed, heavy and red/purple in color, their texture resembling that of liver. Microscopically, the lungs are collapsed, but with many dilated terminal airways. Necrotic bronchial or bronchiolar epithelium is the earliest feature and may be seen before hyaline membranes (HMs) develop (de la Monte et al. 1986). The membranes may become dislodged and plug more distal airways. The eosinophilic HMs lining

the terminal airways are present within an hour or so of birth and often contain nuclear debris of necrotic epithelium (Fig. 20.24). Membranes are not usually seen in the terminal sacs, which are collapsed. After a few hours the presence of polymorphs and macrophages in the interstitium is marked with some spillage into the airways, although the inflammatory changes may not be conspicuous by routine histology (Murch et al. 1996a).

In infants dying at a day or two of age, the lungs show evidence of repair and regeneration. Macrophages ingest membrane (membranophages) but also accompany the fibroblasts proliferating beneath the HMs. Subsequently, regenerating cuboidal epithelium (type 2 pneumocytes) is apparent, often growing over residual HMs, which are incorporated into the bronchiolar walls (Fig. 20.25).

In some cases, the hyaline membranes may be bright yellow, particularly on the luminal surface. Jaundice is not always present but is due to the incorporation of albumen bound bilirubin into the hyaline membranes (Blanc 1976).

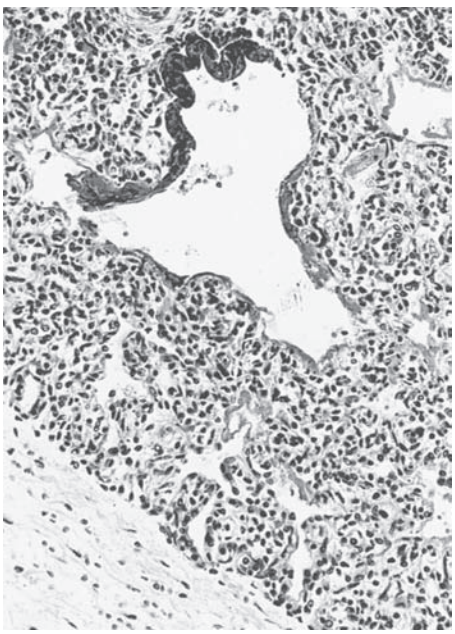

Figure 20.24. Hyaline membrane disease in a 26-week-gestation neonate dying at 12 hours of age. Necrotic respiratory bronchiolar epithelium lines part of a dilated airway. Hyaline membrane is also present at this level and more distally. In the most distal part of the lung, the air sacs adjacent to the pleura are collapsed but do not contain membrane.

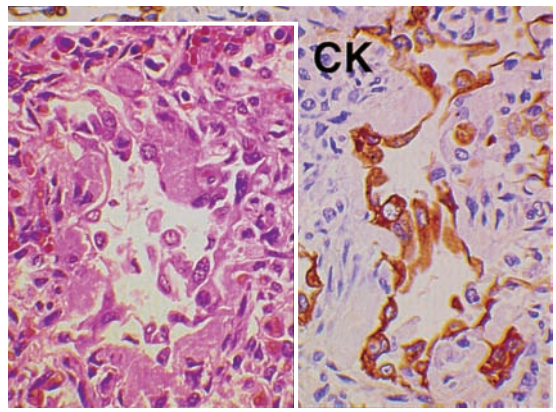

Figure 20.25. Hyaline membranes at 40 hours of age. Reactive macrophages and some fibroblasts are present below the membranes. Immunohistology for cytokeratin (CK) shows regenerating epithelial cells already covering the membranes; some membrane may be incorporated into the wall.

Pathogenesis

Hyaline membranes are composed of necrotic debris, and a proteinaceous precipitate of plasma including some fibrin. For the pathologist, they are the most outward expression of a complex pathophysiological process. The acute transudation from capillaries adjacent to the terminal airways reflects damage to epithelium and endothelium, whose integrity is necessary to form an impermeable barrier between the vasculature and the airways. What produces the damage is not entirely clear. It is presumed that either in the infant's attempts to breathe or because of the positive pressure from the ventilator, the terminal airways expand and shear forces damage the lining epithelium (Robertson 1991). The toxic effect of oxygen might also contribute, or the epithelial damage may reflect a "reflow" injury following a period of local ischaemia. That ischemia may be contributory in some instances is suggested by evidence of increased pulmonary intravascular coagulation (Schmidt et al. 1992). The very terminal parts of the respiratory units, that is, the air sacs, fail to expand because surface tension, which acts to collapse these small "spheres," is too great to overcome in the absence of surfactant.

Surfactant replacement therapy is now recommended for any infant less than 27 to 28 weeks' gestation while slightly more mature infants receive surfactant if their RDS is severe enough to require intubation (Halliday 2003). Its use has seen a major reduction in morbidity and perinatal mortality of up to 65% with no significant complications except perhaps a slight increase in pulmonary hemorrhage, mainly with some artificial replacement surfactants (Halliday 2003). Where infants do die with respiratory distress syndrome, no major difference in the lung pathology between babies who have or who have not had surfactant replacement is described (Pinar et al. 1994; Thornton et al. 1994), although in my experience the membranes tend to be more scant and fragmented than in the natural disease.

Bronchopulmonary Dysplasia (Chronic Lung Disease)

Terms and Definition

The past 20 to 25 years has seen a variable and sometimes less than well defined use of the terms *bronchopulmonary dysplasia* (BPD) and *chronic lung disease* (CLD). Bronchopulmonary dysplasia was described by Northway et al. (1967) in the early days of neonatal intensive care and entailed a lung severely damaged by the therapies used to maintain respiration in the preterm infant. This damage usually occurred in the first few days of life during treatment of an acute lung disease, most commonly the surfactant-deficient hyaline membrane disease of preterm infants. Infants with BPD required intensive ventilatory support and had lungs that showed a spectrum of damage but that commonly included obliterated airways and an interstitial fibrosis. The infants commonly died from respiratory failure or complications directly attributable to fibrotic lungs such as cor pulmonale. With better management and advent of therapies such as surfactant replacement, this pattern of severe lung disease has largely disappeared, but a milder condition has emerged in which continued respiratory support is still required but at a much diminished level. In many cases, the early acute lung disease is relatively mild and there may be an interval between the apparent successful treatment of the acute disease and the onset of a chronic respiratory disease process.

The terms CLD and BPD have been used interchangeably clinically but with CLD gradually becoming the preferred description for this milder pattern of clinical disease, as many neonatologists preferred to avoid the connotations of severe fibrotic lung damage that accompanied the label BPD. However, there are now positive moves to return to BPD (sometimes prefixed by "new") as the preferred term, largely to reduce the potential for confusion between this specific disease of the neonatal period and other chronic lung diseases arising later in life (Jobe and Bancalari 2001; Sosenko et al. 2003; Greenough and Milner 2005).

"New" BPD is almost invariably a clinical diagnosis, and indeed is defined in clinical terms, and describes a baby requiring continued ventilatory support for a prolonged period. It usually follows a period of mechanical ventilation and supplemental oxygen, although there may be a period when no supplemental oxygen is required before oxygen requirements gradually increase and the infant becomes more dependent on ventilatory support. Most babies can eventually be weaned from this support, although a small minority gradually progress and die from respiratory failure. Specific definitions are usually only critical if comparative studies are being made. In babies <32 weeks' gestation, new BPD is classed as mild if oxygen >21% has been required for at least 28 days, but there has been a return to air breathing at 36 weeks postmenstrual age or discharge; BPD is considered severe when there has been a requirement for >30% oxygen at a similar time point. Unlike earlier clinical definitions of BPD, there is now no requirement for cystic changes to be present on chest x-ray or the need for a defined early acute lung disease (Jobe and Bancalari 2001).

For the pathologist, there is one further aspect that has changed while the nomenclature has cycled from BPD to CLD and back to new BPD. Old BPD had very distinctive features that usually allowed a confident pathological diagnosis at autopsy. The new BPD, however is, essentially, a clinical diagnosis based on the period of oxygen dependency. The pathology is far more subtle and is a difficult diagnosis to make on pathological

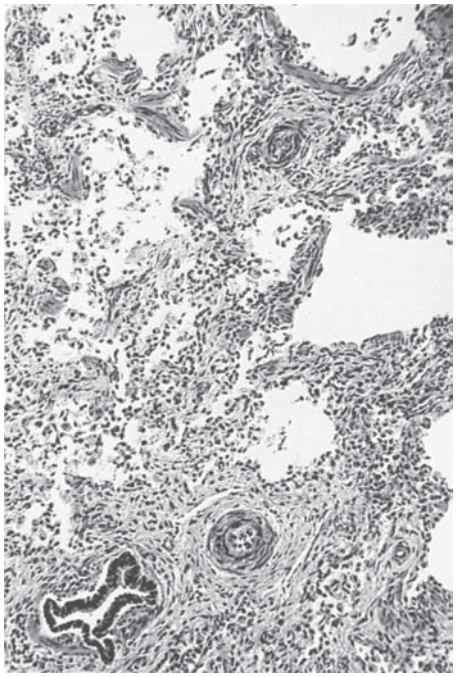

Figure 20.26. Bronchopulmonary dysplasia in an infant dying at 3 months of age. Interstitium shows striking fibrous thickening and the small pulmonary artery is thick walled. The adjacent terminal bronchiole is normal.

grounds alone in the absence of the appropriate clinical setting; indeed, the milder variants are unlikely to be seen by the pathologist as they will not be fatal. The old and the new patterns of BPD will be described in these terms, although it should be emphasized that they still represent a spectrum of lung injury rather than distinct disease entities.

Bronchopulmonary Dysplasia (Old)

The early pathological descriptions of BPD describe lung changes in three phases: an exudative phase from days 3 to 9, a subacute fibroproliferative stage from days 10 to the end of the first month, and a chronic fibroproliferative phase from the end of the first month. In the following descriptions, the more aggressive early phases of BPD barely imply "chronic" lung disease at all; the more florid early stages are now very rarely seen pathologically and this reflects the significantly improved early management of the immature lung:

- *Major airway injury:* Severe bronchial and bronchiolar damage is characterized by necrosis associated with an obliterative bronchiolitis, squamous metaplasia, and collapse of lung tissue distal to the obstructed airway (Bonikos et al. 1976; Taghizadeh and Reynolds 1976). It is a relatively acute phenomenon, with the necrosis occurring in the first few days of life. Its frequency in earlier descriptions of BPD probably reflects the ventilatory management that was then current, and the relatively high inflationary pressures. Severe acute major airway pathology is now highly unusual. Morphometric study suggests that the major persistent airway lesions identifiable are bronchial gland hyperplasia and peribronchiolar smooth muscle hyperplasia (Hislop and Howarth 1989; Margraf et al. 1991)
- *Distal respiratory unit and interstitium:* The most prominent component of the lung injury is a widespread but occasionally patchy interstitial edema and fibrosis (Fig. 20.26) associated with cuboidal metaplasia (Fig. 20.27). Early ventilatory inequality may give rise to areas of relative collapse and fibrosis accompanied by more distended emphysematous lung (Fig. 20.28).

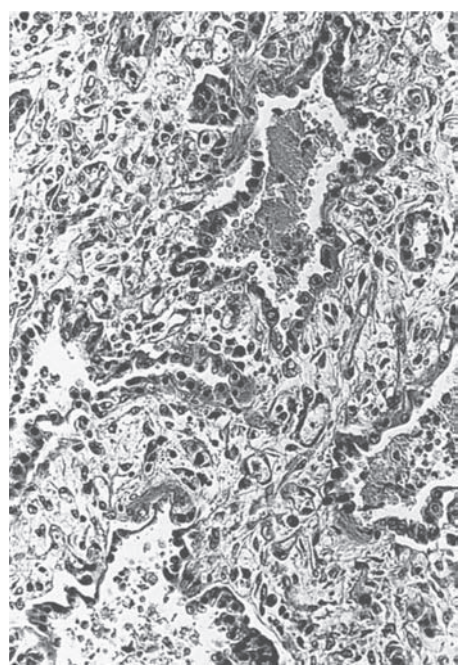

Figure 20.27. Striking cuboidal metaplasia associated with bronchopulmonary dysplasia.

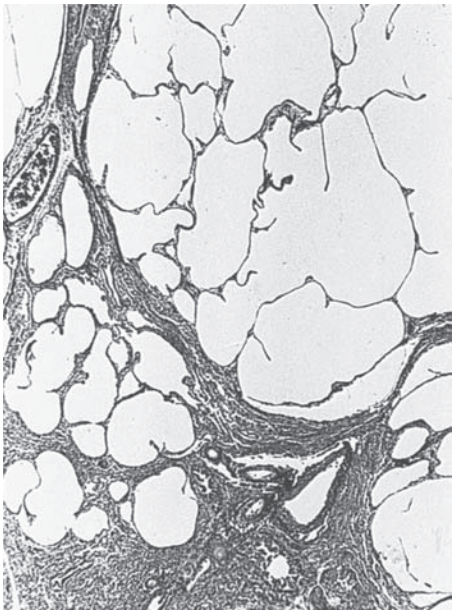

Figure 20.28. Bronchopulmonary dysplasia. An area of emphysema.

Special stains will show increase in the elastic tissue usually in the form of thick plaques at points of bronchiolar or alveolar duct division. The interstitial damage forms a continuum with the repair processes associated with the acute lung injury.

- *Vasculature:* Arterial muscular hypertrophy and adventitial thickening of small pulmonary arteries may come to be the most significant component of the infant with chronic ventilator dependence (Fig. 20.26) (Hislop and Haworth 1990). In some cases, there is evidence of a reduction in peripheral arterial density, possibly due to failure of normal postnatal recruitment (Gorenflo et al. 1991). Eventually, pulmonary hypertension and cor pulmonale develop, although there may not always be good correlation between the occurrence of cardiac complications and the pulmonary histology.

New Bronchopulmonary Dysplasia (Chronic Lung Disease)

Compared with the older pattern of disease, new BPD lungs show little or no major airway damage, and the small pulmonary arteries are relatively normal. Although an increase in elastic tissue can be detected with special stains, interstitial fibrosis also appears minimal or absent. The damage is predominantly due to interference with normal alveolarization so that there are fewer, but larger and simpler, air spaces, leading to a significant decrease in surface area and a reduced abnormal pulmonary microvasculature (Jobe and Bancalari 2001; Thibeault et al. 2004; Galambos and DeMello 2007). Histologically these changes can be relatively subtle, and the relative increase in alveolar size easily missed by the casual observer. There is evidence that interference with the normal processes of septation and alveolarization occurs very early, and the normal collagen framework is affected by ventilation (Thibeault et al. 2003).

Etiology and Pathogenesis

The current and changing incidence of BPD is difficult to specify. It varies markedly depending on gestation and because the pattern of disease and definition of BPD has changed. However, there is some indication that the need for long-term ventilatory support is increasing, possibly because of averted neonatal death from improving therapies (Yu and Ng 1995). Approximately, 20% of very low birth weight babies (<1500 g) still require some supplementary oxygen at 36 weeks postmenstrual age (Sosenko et al. 2003). Clinically, the infants destined to develop BPD are not easy to identify at an early stage, although antenatal factors may be significant. These include early, prenatal lung inflammation (Watterberg et al. 1994; Matsuda et al. 1997). Bronchoalveolar lavage studies indicate a higher level of inflammatory and oxidant markers associated with the acute lung injury in those infants who progress to chronic lung disease (Contreras et al. 1996; Murch et al. 1996b) compared with those who do not.

Additional factors may include the use, too early, of intralipid during parenteral nutrition, which may interfere with oxygenation (Cooke 1991; Stahl et al. 1992); lung colonization with *Ureaplasma urealyticum* (Wang et al. 1995); and antenatal steroids (Jobe 2003).

Bronchopulmonary dysplasia, as a distinct entity, emerged primarily following the introduction of ventilatory support for the preterm infant and resulted from a combination of barotrauma and oxygen toxicity, which interfered with normal growth and repair mechanisms following acute

lung injury. Inflammation is an early response to mechanical injury, and an increase in a wide range of cellular and chemical mediators can be demonstrated (Groneck and Speer 1995; Saugstad 1997). While it is not possible to dissociate entirely the mechanical effects of ventilation from the effects of hyperoxia, high inflationary pressure is likely to be the most significant factor in the obliterative bronchiolar lesions (Taghizadeh and Reynolds 1976; Saugstad 1990) seen in old BPD.

Cellular metabolism produces oxygen-related toxic radicals such as OH^- and O_2^- and singlet oxygen. In the lung, there are probably two main sources of toxic radicals: neutrophils or macrophages as a product of inflammation, and as a by-product of normal pulmonary epithelial or endothelial metabolism. Tissue production of free radicals is enhanced in the presence of high oxygen concentrations and damage is accentuated in the absence of normal cellular antioxidant defenses. The precise mechanism by which tissue damage leads to interstitial fibrosis is unclear, but it may cause an increase in capillary permeability, and fluid leakage with subsequent organization. Platelet-derived growth factor may be produced by the interstitium in response to hyperoxia and stimulate early fibroblast hyperplasia (Han et al. 1992). Inactivation of surfactant may also be important (Merritt 1982; Saugstad 1990; Contreras et al. 1996). There is growing evidence that indicators of oxidative stress can be found very early, possibly only hours after birth (Saugstad 2003).

Air Leaks

The use of positive pressure to maintain lung inflation during hyaline membrane disease may lead to tearing of delicate respiratory tissues. Air leakage into the pleural cavities can be a cause of sudden collapse (Fig. 20.29). Particularly if under tension, a pneumothorax can interfere with venous return and seriously impair cardiac output, causing intracerebral complications. Rapid drainage of air is important, but occasionally drains may damage pulmonary parenchyma.

Pulmonary interstitial emphysema (PIE), when air passes into pulmonary tissues, may track in pulmonary lymphatics and, by compression of adjacent lung, seriously impair ventilation (Fig. 20.30). Clinically, it may produce a dilemma, as excessive ventilatory pressure may exacerbate PIE, whereas the same pressure may be necessary to maintain lung expansion elsewhere. Persistent PIE may loculate and stimulate a giant cell reaction and fibrosis (Fig. 20.31). Often at

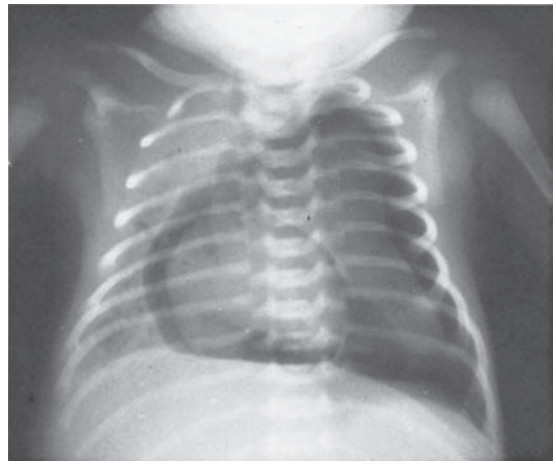

Figure 20.29. Chest radiograph from a baby at 28 weeks' gestation. There is air within the left pleural and pericardial cavities.

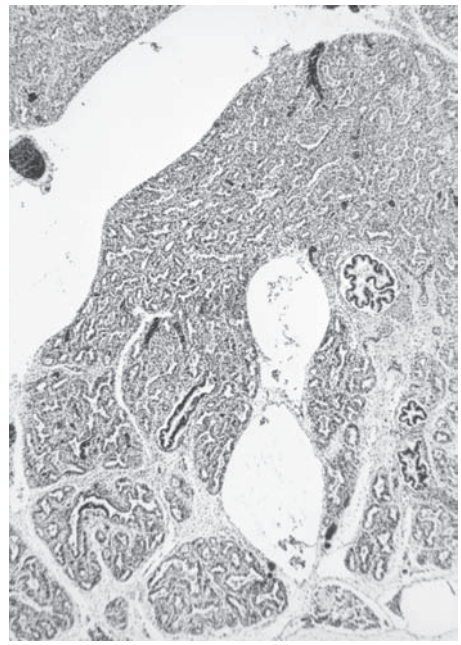

Figure 20.30. Lung from a 25-week early neonatal death showing emphysema and air within septal lymphatics tracking to the subpleural region.

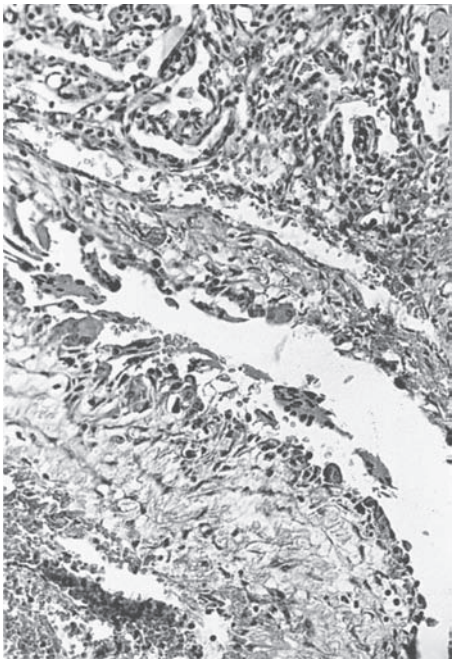

Figure 20.31. Partially collapsed old cyst resulting from interstitial emphysema. Cyst wall is formed of thick fibrous tissue and lined by macrophages including giant cells.

postmortem, due to resorption, PIE is less impressive than the premortem radiographs might lead one to expect. Severe PIE is seen much less frequently with modern therapies especially since the advent of surfactant therapy for HMD. Both pneumothorax and PIE are more likely in the presence of pulmonary hypoplasia.

Pulmonary Hemorrhage

In preterm infants dying during the acute phase of lung injury, some pulmonary hemorrhage is a common finding in the terminal sacs and pulmonary interstitium, especially associated with hyaline membrane disease (Coffin et al. 1993). Hemorrhage may also occur into the dilated pulmonary lymphatics. In some cases massive hemorrhage is a terminal event. It may be associated with birth asphyxia or hemorrhagic disease of the newborn. Although the pathogenesis is not certain, pulmonary hemorrhage may represent a hemorrhagic pulmonary edema (Cole et al. 1973; Amizuka et al. 2003) and reflect terminal heart failure.

Pathology of Birth Asphyxia

Birth asphyxia has been defined as a condition of impaired blood gas exchange leading to progressive hypoxemia and hypercapnia with a significant metabolic acidosis (Low 1997). It typically presents in the term neonate, but may occur in the premature infant and compound the effects of organ immaturity. Evidence of acute asphyxia may also be observed in the lungs of stillborns.

Hypoxia stimulates deep gasping movements allowing movement of amniotic fluid into the airways and more terminal respiratory units (Harding et al. 1990). Amniotic fluid normally contains fetal squames, and these are readily visible in the lungs of stillborns and neonates (Fig. 20.32). A few squames are a common finding and may not be very informative, but large plugs do suggest the occurrence of acute asphyxia, often as a terminal event.

Meconium Aspiration Syndrome

Meconium aspiration syndrome (MAS) is the major respiratory complication of acute asphyxia. Meconium is released from the fetal gut into the amniotic fluid near term in approximately 10% to 15% of infants. It is rarely seen before 34 weeks, and if seen earlier may be associated with infection. Release has been attributed to reflex anal

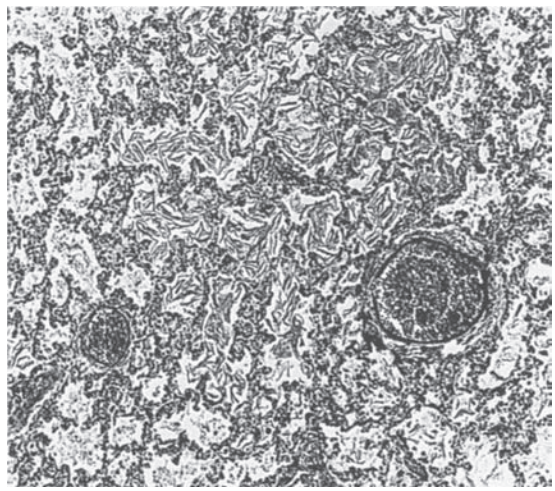

Figure 20.32. Lung from a fresh stillbirth at 37 weeks' gestation. Airways are unexpanded and aspirated squames are present in air spaces.

dilatation from acute hypoxia, but this is disputed (Danielian 1994), and meconium-stained liquor may simply reflect maturity. Only a small proportion of infants born with meconium-stained liquor will exhibit evidence of aspiration, and meconium in the liquor may only be of significance if it is thick and heavily stained.

In neonates, meconium aspiration causes respiratory distress from a number of mechanisms including airway obstruction with distal pulmonary collapse or pneumothorax, inhibition of surfactant (Moses et al. 1991), and predisposition to infection (Romero et al. 1991). Pulmonary hypertension may be a serious complication due, at least in part, to hypoxic vasoconstriction of pulmonary arteries.

There is considerable debate as to the timing of meconium aspiration and its significance in relation to management and prevention (Wiswell et al. 1990; Katz and Bowes 1992; Ahanya et al. 2005). On the basis that aspiration occurred at birth, resuscitation strategies to prevent meconium passage into the lungs have met with limited success, and many authors have stressed that aspiration is an antepartum event associated with evidence of antepartum hypoxia elsewhere such as in the placenta (Thureen et al. 1997). Further, that aspect of MAS due to pulmonary hypertension may reflect antepartum structural changes in pulmonary arterioles resulting from chronic intrauterine hypoxia (Murphy et al. 1984; Thureen et al. 1997).

At necropsy, asphyxia is suggested by petechial hemorrhages, sometimes confluent, on the pleural surfaces of the lungs. The cut surfaces may show congestion and edema. When substantial meconium aspiration has occurred, the lungs are heavy and mottled, and careful inspection may reveal areas of overdistention with yellow–green meconium expressible from airways.

In stillborn infants, acute asphyxia may precipitate amniotic fluid inhalation with substantial plugs of fetal squames throughout the lung (Fig. 20.32). More rarely, meconium are also present as eosinophilic granular material, with small yellowish meconium bodies and mucus (Fig. 20.33). While a few macrophages may be associated with this material, a widespread acute inflammatory infiltrate is unusual, which may indicate that meconium aspiration is often an agonal event in many stillborns. Chronic intrauterine aspiration of meconium associated with lung infarction has been reported (Kearney 1999).

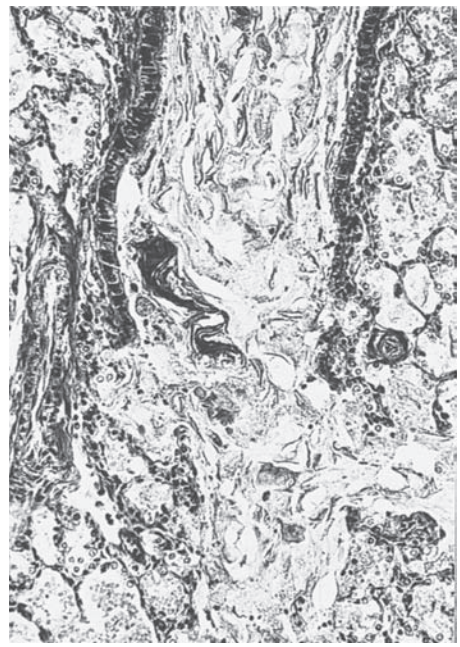

FIGURE 20.33. Terminal bronchiole from term infant containing squames, mucin, and granular material typical of meconium aspiration.

In neonates, meconium aspiration may be accompanied by patchy hyaline membranes associated with the acute hypoxia. If the infant survives the early neonatal period, an acute inflammatory response may develop. There is evidence that meconium causes a chemical pneumonitis possibly due to the bile salt content (Oelberg et al. 1990), but features such as the patchy distribution of the inflammation that accompanies the meconium and the rarity of inflammation in the meconium stained lungs of stillborns suggest that in many situations the pneumonia is due to infection. Typically the vascular changes of pulmonary hypertension are also present (see below).

Persistent Pulmonary Hypertension

Persistent pulmonary hypertension (PPH) of the newborn reflects a failure to reduce or maintain a reduction in the normal postnatal fall in pulmonary vascular resistance. Increased pulmonary vascular resistance may be due to increased

muscularization of the pulmonary arteries, increased vascular reactivity, or decreased vascular bed because of poor lung growth; these factors are not mutually exclusive. Although there may be an initial period of apparent normality, PPH may be indicated by cyanosis and respiratory distress, which may develop after birth; the associated hypoxemia responds poorly to supplemental oxygen. There is right-to-left intrapulmonary shunting but also across the foramen ovale and the ductus arteriosus (if it remains patent). Because of this latter feature, it has been referred to as persistent fetal circulation (Gersony 1973).

Pulmonary hypertension may be associated with other abnormalities such as congenital heart disease or congenital alveolar capillary dysplasia (see above). Late presentation may occur rarely in babies with trisomy 21 even in the absence of a relevant structural cardiac defect. A more common association is lung hypoplasia, where there is a failure of the normal development of the pulmonary circulation and a reduced vascular bed. Other mechanisms may also be involved (Doolin et al. 1995). It may also occur in association with sepsis, pneumonia, hyperviscosity, acute hypoxia with meconium aspiration, and hypoglycemia. Rarely it may be idiopathic, although it has been argued this does not exist (Perlman et al. 1989).

Idiopathic PPH and the hypertension associated with MAS may be associated with vascular changes of prenatal origin, possibly due to intrauterine chronic hypoxia. The preacinar and intraacinar pulmonary arteries and arterioles show medial hyperplasia with extension of smooth muscle into precapillary vessels (Murphy et al. 1984; Raine et al. 1991; Thureen et al. 1997). Nerve fibers have been found at a more distal location than normal (Raine et al. 1991).

Infection

The newborn infant is susceptible to infection, particularly if very preterm or suffering from growth restriction. Infection may be acquired in utero, intrapartum, or in the neonatal period, and while the lung may not always demonstrate the most significant pathology, pulmonary involvement is frequent. In utero, transmission to the fetus is by two main routes: ascending through the maternal uterine os and transplacentally from the maternal circulation (Zaaijman et al. 1986).

Ascending Infection

Abortion and Stillbirths

Evidence of an ascending infection is a common feature of spontaneous abortions particularly before 24 weeks' gestation, and chorioamnionitis may be found in up to 20% to 30% of cases. There are often no specific macroscopic features, although effusion, which may be blood stained, may be associated with infection in stillborns. It should not be mistaken for the effusions associated with maceration.

Microscopically, the fetal lung may show a few polymorphs within the airways only, or the infiltrate may be sufficient to be designated a pneumonia. Acute interstitial reaction is usually present but less obvious histologically. Studies have shown the airway cellular reaction is fetal in origin, not aspirated maternal cells as formerly believed (Grigg et al. 1993). A more chronic reaction may occur in the interstitium with both hemopoietic cells and lymphocytic aggregates (Fig. 20.34). The latter, which is typically closely applied to the bronchial epithelium, has parallels with the Peyer's patch of the gut. These infiltrates are probably always a reaction to ascending infection even if an acute reaction is not detectable (Gould and Isaacson 1993; Sgrignoli et al. 1994).

The infecting organism may be a common gastrointestinal commensal such as *Escherichia coli* or a vaginal commensal such as group B streptococcus (GBS). *Candida* infection may produce superficially nonspecific but usually very florid pneumonia in which the characteristic hyphae can be seen (Fig. 20.35). Frequently, especially in abortuses, no organism is cultured despite florid histological evidence of infection. *Mycoplasma*, *Ureaplasma* or even *Chlamydia* (Gravett et al. 1986; Lamont et al. 1987; Cassell et al. 1993) may be involved in a proportion of cases.

Neonate

Neonatal infections are frequently divided clinically into early or late onset. Early-onset pneumonia reflects an ante- or intrapartum acquired organism. Symptoms usually start within a few

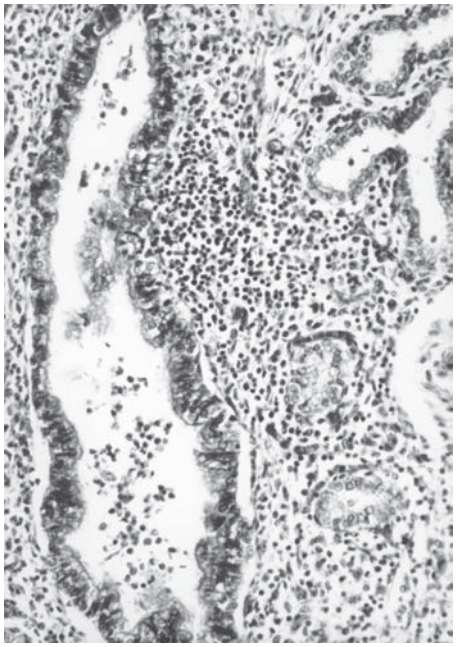

Figure 20.34. Ascending infection in lung from 20-week-gestation spontaneous abortion. A few polymorphs are visible in the airway and evidence of a more chronic response is present with an adjacent interstitial lymphocytic aggregate.

hours after birth but can be delayed for up to 48 hours. Most bacterial infection produces a typical nonspecific bronchopneumonia, although some organisms produce characteristic features. Group B streptococcus, normally an innocuous vaginal commensal, is a particularly virulent organism in the neonate. Death may be very rapid following acute collapse and may mimic perinatal asphyxia. Developing pulmonary infection may cause respiratory distress, indistinguishable clinically and radiographically, from surfactant deficient hyaline membrane disease (Ablow et al. 1976). Subsequent problems include pulmonary hypertension, reduced cardiac output, and systemic hypotension. Early GBS infection is not invariably associated with evidence of ascending infection in the placenta (De Paepe et al. 2004). Many of the cardiovascular effects of GBS may be mediated by tumor necrosis factor (Gibson et al. 1991). Although usually sensitive to antibiotics, deterioration and death from GBS may supervene before effective control has been achieved.

If onset of infection and death are very rapid, the lungs may show surprisingly little by way of pathology other than congestion and edema. Small patches of inflammation within major airways may be suggestive, and careful examination with a Gram stain may reveal a few organisms. Longer survival is associated with the more typical picture of bronchopneumonia, although hyaline membranes, often containing abundant stainable organisms giving a blue tinge to the membranes, may dominate some areas (Fig. 20.36).

The pathology of late-onset pneumonia is similar to that of early onset but with a different range of organisms. *Pseudomonas* is a frequent

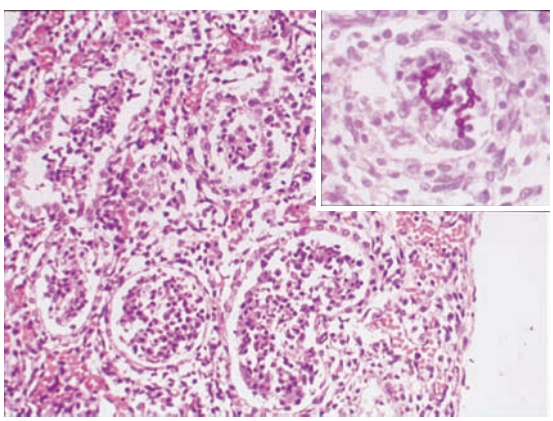

Figure 20.35. Florid pneumonia associated with ascending infection in a 17-week-gestation fetus. Inset shows typical *Candida* (periodic acid-Schiff stain).

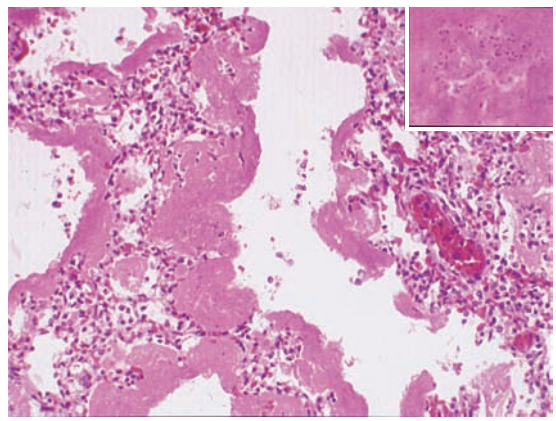

Figure 20.36. Dense hyaline membranes in term infant dying at approximately 12 hours of age from overwhelming group B streptococcal infection. Under high power (inset) bacteria are visible in the membrane.

colonizer of neonates but can cause a severe pneumonia. Histologically, areas of more typical pneumonia may be associated with striking growth of the organism in vessel walls (Bonifacio et al. 2003). Hemorrhage and infarction can result from vessel thrombosis (Teplitz 1965). *Proteus* may do the same.

Other bacterial infections with characteristic appearances include *Listeria monocytogenes*. Involved as part of disseminated disease, the lung may show the typical granulomatous abscesses involving parenchyma and vessels as seen in other tissues (Vawter 1981; Khong et al. 1986). Congenital syphilis is not common in most developed countries, but should be considered as a cause of a congenital pneumonia where there are interstitial infiltrates of lymphocytes, plasma cells, and "onion-skinning" of pulmonary arteries (Oppenheimer and Dahms 1981). This "pneumonia alba" is usually only one manifestation of the disease, and histological evidence of syphilis is likely to be present elsewhere such as in the liver or pancreas.

Fungal Infection

Pulmonary *Candida* occurs either as a manifestation of an ascending infection and amniotic fluid infection or as a component of systemic candidiasis (Keller et al. 1977; Kassner et al. 1981; Whyte et al. 1982). In the former case, the infection is a typical bronchopneumonia in which fungal hyphae can be identified (Fig. 20.35). In systemic infection, the vasculature is usually the prime site of infection from septic emboli.

Viral Infection

Most viral infections can affect the lungs, but other tissues or organs often demonstrate more characteristic or extensive damage. Particularly in abortuses or stillbirth, however, pulmonary histology is often better preserved than elsewhere and useful to detect parvovirus.

Congenital cytomegalovirus (CMV) may produce a pulmonary interstitial infiltrate of mononuclear cells associated with the typical inclusions in macrophages and epithelial cells. More commonly, CMV is encountered in infants who have been long-term residents of the neonatal unit, having required ventilation for chronic lung disease (Fig. 20.37). The inclusions may be sparse, and in the context of some cases it may be difficult to determine the contribution of the CMV to the lung damage.

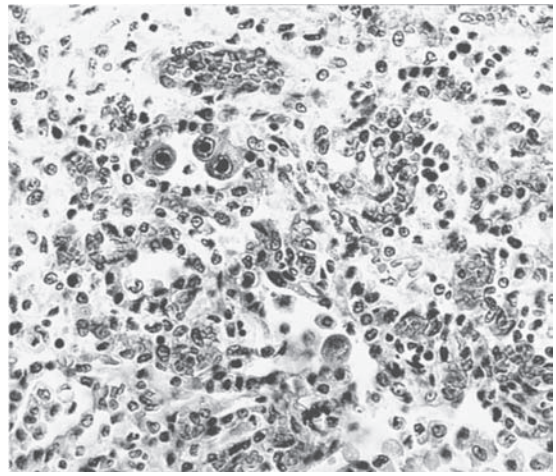

Figure 20.37. Cytomegalovirus inclusions in the lung of an infant dying from chronic lung disease. The infection is not congenital and was acquired while the infant was on the neonatal unit.

Herpes simplex virus may be acquired antenatally or during passage through the birth canal. Babies typically present at the start of the second week of life. The organs most typically affected and showing virally induced necrosis are the liver and adrenals, but the lung also may be involved. The lung may appear normal macroscopically or show small white necrotic foci. Microscopically, there may be a pneumonitis or hyaline membrane disease. The necrosis is often bland and the extent of the viral infection not readily apparent unless specific immunohistology performed (Fig. 20.38).

Other viruses are generally very rare in the neonatal period. Respiratory syncytial virus (RSV) and metapneumovirus (Ulloa-Gutierrez et al. 2004) are normally seen in older infants, although RSV may be found in neonates in association with chronic lung disease or congenital heart disease (Hall et al. 1979). Features include bronchiolar plugging with mucus, epithelial desquamation, inflammatory changes, and giant cells lining distal airways. Enteroviruses, including Coxsackie and echovirus subtypes, have all been reported as causing an acute neonatal pneumonitis probably acquired during birth from maternal secretions

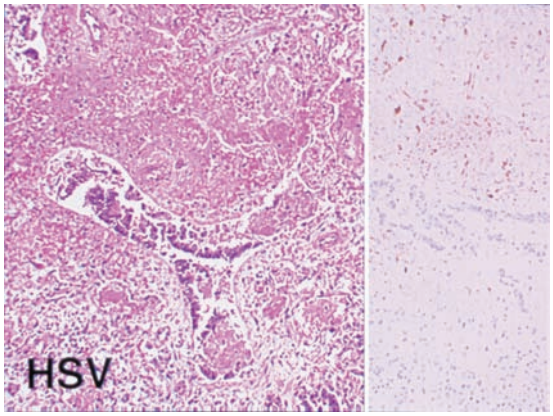

Figure 20.38. Herpes simplex virus (HSV) (type 2) in lung from an infant with disseminated disease. Inclusions are difficult to find, the necrosis is bland, and there is little associated inflammatory reaction. Immunohistology shows the presence of viral antigen confined mainly to the areas of necrosis.

(Modlin 1986; Abzug et al. 1990). The illness may be very severe and resemble a bacterial pneumonia clinically. There is a case report implicating a coronavirus associated with chronic lung disease (Sizun et al. 1994).

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The Cardiovascular System

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In the past few decades there has been a great expansion in our knowledge of fetal and neonatal heart disease. The application of the techniques of molecular biology to the investigation of the developing embryo has revolutionized our understanding of the formation of the heart and has given great insights into the genes involved in its control. The development of the normal heart and its deviations from normal in disease are dynamic processes that change throughout pregnancy and postnatal life. The use of high-resolution echocardiography has provided previously undreamed of insights into normal physiological processes both in utero and after birth. The increasingly good results of pediatric cardiac surgery and the possibility of cardiac transplantation have given hope for conditions that were previously hopeless. In addition to all this, the careful longitudinal study of heart defects in utero has allowed us to chart the natural progression of lesions before birth and raises the tantalizing possibility of in-utero therapy before secondary changes convert a bad situation into a hopeless one. Now, more than ever, it behooves the pathologist to understand cardiovascular pathology from the stage of heart development to the postnatal period, and a close working relationship with colleagues—cardiologists, surgeons, fetal medicine specialists, and geneticists—is essential if the optimal diagnostic and treatment goals are to be realized for this group of patients and their families.

Development of the Heart

Much of the detail of the early development of the heart is based on studies in chicken and mouse embryos (Tam and Schoenwolf 1999), supplemented by morphological examination of early human embryos.

The Heart Fields

The heart develops from asymmetrical bilateral plates of cardiac mesoderm that express the cardiac specific genes *Nkx2.5* and *GATA4* (Olson and Srivastava 1996). These plates form a primitive heart tube, lined by endocardium with a surrounding sleeve of myocardium, both layers separated by extracellular matrix termed cardiac jelly. The inflow is caudal and the outflow cranial. The myocardium shows regular contractions by the third week. The heart tube undergoes looping and septation during which the arteries, veins, atria, and ventricles are delineated. Heart formation is completed with the development of valves and the conduction system and the formation of the coronary arteries by the ingrowth of extracardiac tissues derived from the neural crest and from the proepicardium situated in the septum transversum (Gittenberger de Groot et al. 2005).

Looping of the Heart Tube

The straight heart tube already shows patterns of gene expression that determine the fate of its

various parts (Kirby 2002). Traditionally, five consecutive segments are recognized: venous, atrial, left ventricular, right ventricular, and arterial. Between each of the segments is a transition zone, the sinoatrial ring, the atrioventricular canal, the primary heart fold, and the ventricular outflow tract. It is important to recognize that the cardiac chambers develop as outgrowths from these segments and that the original segments contribute more to the connections of the cardiac chambers than to the chambers themselves. Looping of the straight heart tube begins following activation of a gene cascade that determines right-left symmetry. These genes are already expressed in the cardiac mesoderm before formation of the heart tube and include the genes *lefty*, *nodal*, and *Pitx2*, of which *Pitx2* is the controlling gene (Brown and Anderson 1999; Harvey 2002). Looping is caused by growth of the tube by addition of cells at each end, with those at the caudal end deriving from the primary heart fields and those at the cranial end deriving from the secondary heart field, and by dissolution of the tethering dorsal mesocardium. The direction of looping is not random but is controlled by genes not yet understood and is not under the control of *Pitx2*. The tube loops to the right, and the looping causes all four transition zones to be brought into close proximity on the inner curvature of the loop. This is absolutely essential for establishing the correct connections of the chambers (Gittenberger de Groot et al. 2005).

Development of the Chambers and Septation

The atria and ventricles develop by ballooning growth from the heart tube—the atria from the dorsal aspect and the ventricles from the ventral aspect (Moorman and Christoffels 2003). In the atrial segment this growth is bilateral and in parallel, whereas in the ventricular segments it is unilateral and in sequence. Thus, in the atria it is possible to develop isomerism, whereas in the ventricles it is impossible. The endocardial cells of the atrioventricular canal and outflow tracts give rise to cells that populate the cardiac jelly to form cardiac cushions that contribute to the septation of these regions. At the same time the atria and ventricles develop trabeculations. The myocardium that forms the chambers shows specific gene expression (*Anf, Cx40, Cx43*) but does not show *Tbx2* and *Tbx3* expression, which are repressors of the process and persist in the primitive myocardium of the primary heart tube (Moorman and Christoffels 2003).

Initially, because the atrial segment is connected to the left ventricular segment, there is no direct connection with the right ventricle, but blood can flow from the atria to the right ventricle via the interventricular foramen. This connection is established by growth of the ventricular inlet to the right. Likewise the outlet is connected initially solely to the right ventricle, but by a similar differential growth the outlet comes to overlie both ventricles. The ventricular septum grows from the apex of the heart loop between the left and right ventricular segments. The atria incorporate the draining veins and form a pair of valves around the sinus venosus. Fusion of the anterior part of these valves creates the septum spurium, which contributes to closure of the atria. The primary atrial septum develops to the right of this. An opening then forms in the primary septum—the ostium secundum—and this is partly closed by growth of the septum secundum. The ostium primum is actually beneath the free edge of the septum primum and is obliterated when that edge fuses with the endocardial cushions and the tip of the septum spurium. The pulmonary vein enters the left part of the atrium and is incorporated into it. The exact site of development of the pulmonary vein is still controversial (Moorman et al. 2003).

During looping, the cardiac jelly is eliminated from much of the heart tube but persists at the site of the endocardial cushions at the atrioventricular junction (Person et al. 2005). These form superiorly and inferiorly and fuse in the midline to create right and left atrioventricular orifices, differential growth of the right atrioventricular canal having brought the right atrium into contact with the right ventricle. Fusion of the cushions with the developing interventricular muscular septum and the atrial primary septum completes septation of the atrial and ventricular chambers (Fig. 21.1).

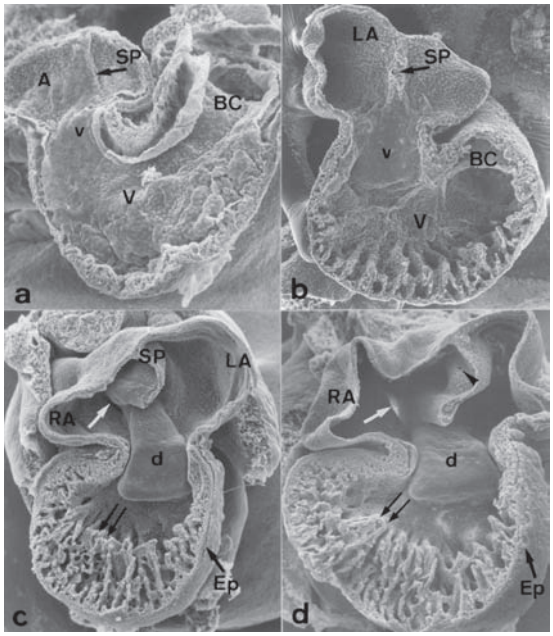

Figure 21.1. Scanning electron micrographs of chick embryo hearts depicting some of the progressive changes that take place in the inner surface of the heart. The hearts have been dissected frontally. (A) Third day of incubation; ventral half of the specimen. (B) End of the third day of incubation; ventral half of the specimen. (C) Four and a half days of incubation; dorsal half of the specimen. (D) Fifth day of incubation; dorsal half of the specimen. The primitive atrium (A) appears, separated from the ventricle (V) by the atrioventricular canal (A-V). The development of the septum primum (SP) divides the primitive atrium into right (RA) and left (LA) atrial chambers. The septum primum grows caudally toward the cushions of the atrioventricular canal. The foramina that open in the septum primum are indicated by the arrowhead in (D). The lumen of the atrioventricular canal is progressively occupied by the growing dorsal (d) and ventral (v) endocardial cushions. The bulbus cordis (BC) constitutes the prospective outflow tract region of the heart. The inner surface of the primitive ventricle (V) is modified by the development of the trabeculae. The single ventricle is divided into two chambers by the interventricular septum (large black arrow in C and D). The interventricular septum appears to develop by coalescence of the primitive trabeculae near the ventricular apex. The sinus venosis (the prospective coronary sinus) opens (white arrow in C and D) into the dorsal wall of the right atrium. The epicardium (the prospective visceral pericardium) (EP) appears in C and D. (From Manasek et al. 1988.)

The outflow tracts have a complex origin, partly from the primary heart tube and partly from ingrowth of cells from two distinct sources. The first of the sources is the cardiac neural crest. The second is the secondary cardiac plate, a region of cardiac mesoderm close to, but distinct from, the primary heart plates. The complex interaction of all these tissues gives rise to the ventricular outflow tracts, the arterial valves, and the intrapericardial parts of the aorta and pulmonary trunk (Anderson et al. 2003).

The pericardium develops as a sac around the developing heart tube. Initially the tube is connected to the posterior mediastinum by the dorsal mesocardium, but this breaks down, permitting the folding of the heart tube on which subsequent development is so critically dependent. The atrioventricular valves develop from the two endocardial cushions.

The coronary arteries and veins develop by both vasculogenesis (the formation of vessels in situ) and angiogenesis (the formation of new vessels by sprouting from existing vessels) from cells that grow over the myocardium form the proepicardium. Both the endothelium and the medial smooth muscle of the coronary arteries derive from this source. These vessels link up and grow to join with the aorta.

The conduction tissue develops from the myocardium of the primitive heart tube, being found in the transitional zones. The formation of an insulating fibrous and fatty tissue plane between atrial and ventricular myocardium occurs only after completion of septation, beginning at 7 weeks and largely complete by 12 weeks of development (Wessels et al. 1996).

The Fetal Circulation and Changes at Birth

The Ductus Venosus and Foramen Ovale

In the fetus, blood is oxygenated by the placenta and returned to the fetus via the umbilical vein, which joins the portal vein at the hepatic hilum. It supplies, preferentially, the left lobe of the liver and bypasses much of the right lobe via the ductus venosus, to enter the inferior vena cava. The proportion of umbilical venous blood that passes through the ductus venosus varies markedly from 20% to 90%. Blood flow velocity in the ductus venosus is 65 to 75 cm/s, whereas abdominal vena

caval flow velocity is approximately 16 cm/s. Thus there is streaming of inferior caval blood as it enters the right atrium. The anterior stream is slower and with poor oxygen saturation, being derived from the inferior vena cava and right hepatic vein. The posterior stream from the ductus venosus is faster and has a much higher oxygen saturation. Blood from the ductus venosus is preferentially directed to the foramen ovale by the eustachian valve and its higher velocity. The greater part of the more anteriorly placed bloodstream derived from the inferior vena cava crosses the tricuspid valve. The deoxygenated superior caval blood is directed preferentially to the tricuspid valve and right ventricle. Only about 5% of this stream crosses the oval foramen (Rudolph 2001b).

Ductus Arteriosus, Lungs, and Systemic Circulation

The right ventricle pumps blood into the pulmonary trunk. Because of the high pulmonary resistance, less than one third of the right ventricular output goes to the lungs and over two thirds passes via the ductus arteriosus to the descending aorta (75 mL/kg/min and 175 mL/kg/min, respectively, in the late-gestation fetus). The left atrium receives its blood from across the oval fossa and from the pulmonary veins. This blood flows across the mitral valve and is ejected by the left ventricle into the ascending aorta, where it supplies preferentially the head and upper limbs, only about one quarter of the left ventricular output crossing the aortic isthmus to join the blood flowing through the ductus arteriosus into the descending aorta. The ratio of right to left ventricular output is on the order of 1.2:1 to 1.3:1, equivalent to 200 to 250 mL/kg/min in the late gestation fetus.

Unlike the situation in the adult circulation, there is mixing of oxygenated and deoxygenated blood at several points in the fetal circulation: in the liver, in the inferior vena cava, and in the left atrium. Streaming of blood partly prevents mixing by preferentially directing oxygenated blood through the oval fossa to the left heart and hence to the head and by directing inferior and superior caval blood to the right ventricle and via the ductus arteriosus to the descending aorta and from thence to the placenta (Rudolph 2001a) (Fig. 21.2).

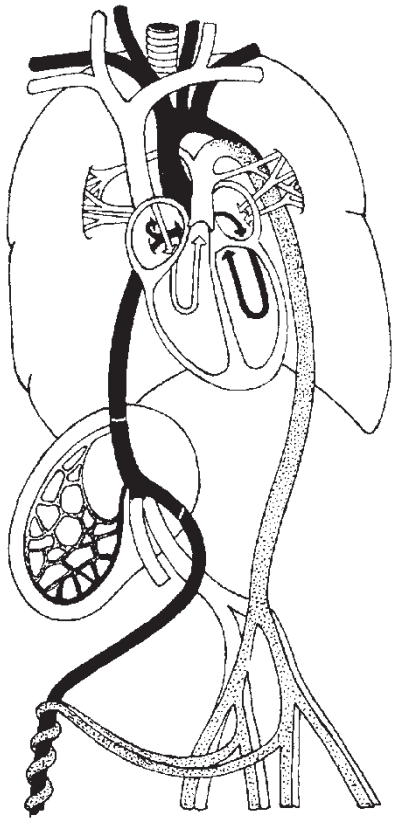

Figure 21.2. The fetal circulation. *Black shading*, oxygenated blood at high PO_2; *stippled shading*, desaturated blood at relatively low PO_2. (From Rigby and Shinebourne 1981.)

Postnatal Adaptation

At birth, the function of oxygenation of the blood, previously subserved by the placenta, is assumed by the fetal lungs. With its first gasps, the newborn expands its lungs with air, resulting in a reduction in pulmonary vascular resistance. This continues in the immediate postpartum weeks by remodeling of the pulmonary vascular bed. This drop in pulmonary vascular resistance has several effects. It causes redistribution in blood flow from the right ventricle. Instead of passing preferentially through the ductus arteriosus, blood passes to the lungs. This increases pulmonary venous return to the left atrium with a subsequent rise in left atrial

pressure. This rise in pressure pushes the flap valve of the oval fossa tight against the septum and seals the interatrial communication. The rise in arterial oxygen saturation brought about by breathing causes the ductus arteriosus to contract and to become functionally closed by 10 to 15 hours after birth. Consolidation of this closure takes place over the following days by fibrosis. The ductus venosus closes at about 4 days of age in the term neonate, but closure takes up to 2 days longer in the premature infant and in those given antenatal steroids (Kondo et al. 2001).

At birth, the pulmonary trunk and aorta have an identical histological appearance. Following the drop in pulmonary arterial pressure associated with decreased pulmonary resistance, the wall of the pulmonary trunk becomes thinner because of loss of smooth muscle, and there is fragmentation of its elastic lamellae, such that by several months of age the histological appearances of the aorta and pulmonary artery are quite distinct.

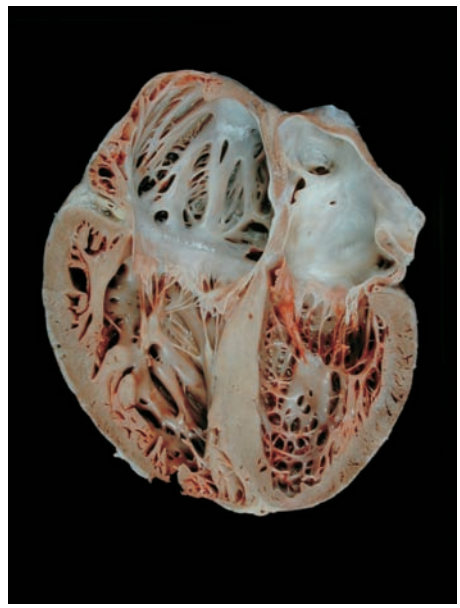

Figure 21.3. A normal heart cut in a simulated four-chamber echocardiographic view. Note the smooth lining of the left atrium (left upper) and the extension of muscular trabeculations around the wall of the right atrium (right upper). The right ventricular apex (lower right) shows coarse muscular trabeculations and the left ventricular apex (lower left) shows fine criss-cross trabeculation. Note also the chordal attachments of the septal leaflet of the tricuspid valve to the interventricular septum; there are no chordal attachments of the mitral valve leaflets to the septum.

Examination of the Normal and Malformed Heart

Normal Anatomy

Knowledge of the anatomy of the normal heart is essential before tackling an abnormal heart!

The Right and Left Atrium

The right atrium comprises three components: a smooth walled venous component, an atrial appendage, and a vestibule supporting the tricuspid valve.

The venous component is smooth-walled and lies between the orifices of the superior and inferior caval veins and encompasses the orifice of the coronary sinus. Embryologically it derives from the sinus venosus and is separated from the atrial appendage externally by the terminal groove and internally by the terminal crest (crista terminalis).

The right atrial appendage is triangular in shape and has a broad junction with the atrium (Fig. 21.3). Its pectinate muscles extend around the greater part of the orifice of the tricuspid valve and are limited by the terminal crest. The junction of the crest of the appendage with the superior vena cava marks the site of the sinoatrial node.

The vestibule supports the tricuspid valve and is smooth. Situated in the vestibule, between the orifice of the coronary sinus, the attachment of the tricuspid valve, and the membranous septum (triangle of Koch), lies the atrioventricular node. The bundle of His exits the node anteriorly to penetrate the membranous septum and divide astride the crest of the interventricular muscular septum giving rise to the right and left bundle branches.

The interatrial septum is smaller than it appears. The true septum comprises only the oval fossa with its rim. The remainder of the party wall with the left atrium is formed by the infolding of both atrial walls, with a layer of extracardiac adipose tissue in between. The oval fossa is closed by a flap valve. In about 20% of the population the

valve is probe patent at its anterosuperior margin (sometimes termed persistent foramen ovale).

The right atrium contains a eustachian valve of variable prominence, a relic of the structure that in fetal life directed the ductus venosus blood from the inferior vena cava through the oval foramen. The coronary sinus may be guarded by a thin valve, the thebesian valve, which may be fenestrated.

The left atrium is usually smaller than the right and receives the pulmonary veins. Usually there are four—two on the right (one superior and one inferior) and two on the left (one superior and one inferior)—but the number can vary. The left side of the oval fossa is generally corrugated and rougher than on the right. The endocardium of the left atrium is thicker than that of the right atrium, the thickening being caused by fibroelastic tissue. This should not be mistaken for a pathological change. The left atrial appendage is quite distinct from the right. It is long and tubular and has a narrow junction with the atrium and characteristically has a hooked extremity. Pectinate muscles are confined to the appendage and do not extend onto the atrial wall nor around the orifice of the mitral valve.

The Ventricles

The ventricles have three components: an inlet comprising the atrioventricular valve and its supporting structures, an outlet supporting the arterial valve, and the apical trabecular component linking the two. The apical trabecular component is the most constant and most characteristic feature of the ventricles. On the right side the septal aspect of the apex shows thick muscle bundles termed trabeculations (trabeculae) that have a roughly parallel orientation along the long axis of the septum (Fig. 21.3). The most prominent of these, the septomarginal trabeculation (trabecula septomarginalis), extends nearly the full length of the septum. Shaped like the letter Y, its stem extends from the apex upward, its anterior limb extends in the outflow tract to the pulmonary valve, and its posterior limb extends backward, supporting the medial papillary muscle of the tricuspid valve. On the left side of the septum the trabeculations are fine and typically have a criss-cross appearance. The outflow tract of the left ventricle shows a smooth septal surface.

The tricuspid valve, as its name indicates, has three leaflets: septal, anterosuperior, and inferior. All three leaflets are anchored by fibrous cords (chordae tendineae) to papillary muscle groups situated at the leaflet commissures. The septal leaflet is attached by cords to the septum. Its medial papillary muscle is small and attached to the posterior limb of the septomarginal trabeculation and marks the site of the artery to the atrioventricular node. The anterosuperior leaflet is the large and its anterior papillary muscle is prominent. The inferior leaflet is less conspicuous, as are its papillary muscles.

The mitral valve comprises two leaflets: a large rectangular, anterior (or aortic) leaflet, and a mural leaflet, which is attached to about two thirds of the atrioventricular junction. Two large papillary muscle groups, termed anterolateral and posteromedial, support the leaflets. The anterior leaflet is attached to the interventricular septum only on its posteroinferior aspect; the left ventricular outflow tract is interposed between the ventricular aspect of the leaflet and the septum, and there is fibrous continuity between the anterior leaflet and the aortic valve cusps. The attachment of the mitral valve to the septum is higher than the attachment of the tricuspid valve to the septum, a feature easily detected on echocardiography and useful for identifying the ventricles (Fig. 21.3). This also means that there is an area between the two attachment sites where there is a potential communication between the left ventricle and the right atrium—the so-called atrioventricular septum.

The septum between the two ventricles is predominantly muscular but, in its upper part, there is a small fibrous area situated between the right and noncoronary cusps of the aortic valve and extending beneath the noncoronary cusp; here, the septum is very thin and completely fibrous—the membranous septum. On the right side, the attachment of the tricuspid valve runs diagonally across this membranous septum. The membranous septum may be abnormally large in trisomy 21 (Rosenquist et al. 1974).

The left ventricle is the thicker walled of the two and is ellipsoid in shape. The right ventricle is wrapped around it, thus giving the right ventricle

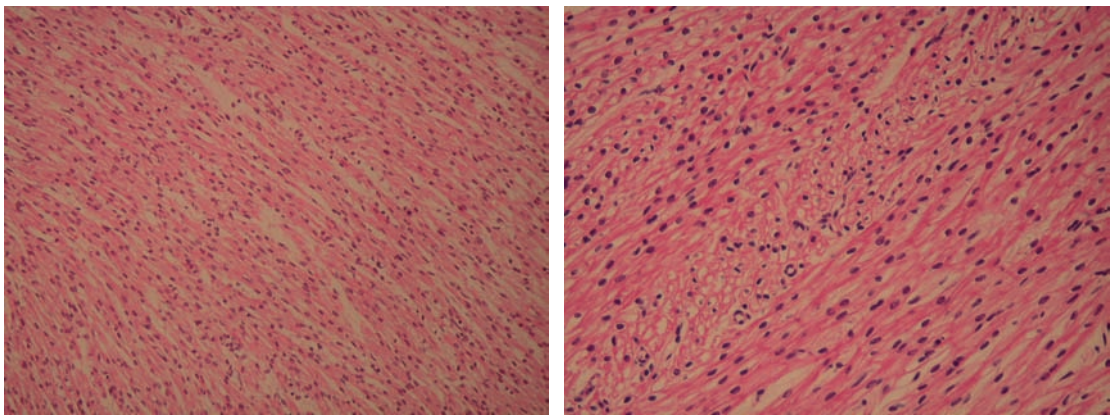

Figure 21.4. (A) Fetal ventricular myocardium at 20 weeks' gestation. The fibers are small and closely packed and show a greater degree of cellularity than is present in adult myocardium. (B) Fetal ventricular myocardium at 40 weeks' gestation. Fetal myocardium shows perinuclear clearing that should not be misinterpreted as evidence of a storage disorder.

a more complex shape. The pulmonary artery arises from the right ventricle and the aorta from the left. The aortic valve sits in the center of the base of the heart and is wedged between the tricuspid and mitral valves. The pulmonary valve sits anteriorly and to the left of the aortic valve. Both valves have three cusps and the commissure of two of the cusps of the pulmonary valve is contiguous with the commissure of two of the cusps of the aortic valve to produce facing sinuses in each valve. The two facing sinuses of the aortic valve give rise to the coronary arteries. The pulmonary valve is supported by a complete muscular infundibulum; the aortic valve, by contrast, has a rim supported only partly by muscle and partly by the fibrous continuity between the left coronary and noncoronary leaflets of the aortic valve and the anterior leaflet of the mitral valve.

The aorta and pulmonary trunk have a spiral relationship with each other—the pulmonary trunk extending backward and to the right and the aorta slightly forward and to the left.

Histologically, the fetal myocardium appears more vacuolated than its adult counterpart (Fig. 21.4).

The Abnormal Heart

Assessment of the abnormal heart is straightforward in the vast majority of cases, provided that some simple rules are followed. The near-universally followed method of examining and describing the heart relies upon sequential segmental analysis (Van Pragh 1972). This system recognizes the heart to comprise three segments:

Atrial segments
Ventricular segments
Arterial segments

These segments are identified by their most consistent feature. For the atria this is the morphology of their appendages, for the ventricles it is the morphology of their apical trabecular component, and for the arterial trunks it is the presence of the coronary arteries. The atrial situs is determined and, following identification of the segments, segmental morphology is noted and intersegmental connections are assessed. Associated abnormalities can then be described.

Situs applies only to the atria; the atrial situs can be solitus (usual arrangement), inversus (reversed), or isomeric (mirror image). Where there is atrial situs solitus, there is a morphologically right atrium on the right side and a morphologically left atrium on the left side. In situs inversus, the morphologically left atrium is situated on the right side and the morphologically right atrium on the left side. In isomerism, both atria are of similar morphology, either both of right-sided morphology or both of left-sided morphology.

The connection of the segments is assessed both at the atrioventricular level and the ventriculoarterial level. The connection at either of these levels can be concordant, discordant, or show absence of connection. Additionally, at the atrioventricular level there may be ambiguous connection or double inlet to one ventricle; at the ventriculoarterial level there may be double outlet from one ventricle. With concordant atrioventricular connection the morphologically right atrium is connected to the morphologically right ventricle and the morphologically left atrium to the morphologically left ventricle.

In discordant atrioventricular connection the morphologically right atrium is connected to the morphologically left ventricle and the morphologically left atrium to the morphologically right ventricle. In discordant ventriculoarterial connection the aorta arises from the right ventricle and the pulmonary trunk from the left ventricle.

Ambiguous connection relates to the atrioventricular connection in atrial isomerism, whether left or right. Self-evidently, one atrium is going to be connected to the right ventricle and one to the left, but since both atria are of the same morphology, the connection cannot be described as concordant or discordant and must therefore remain ambiguous.

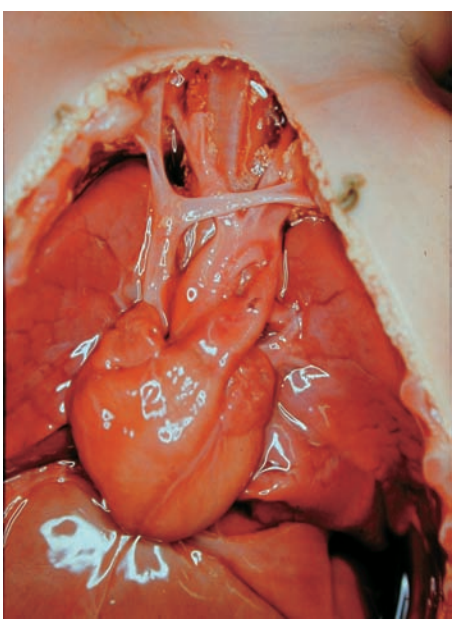

Figure 21.5. Normal fetal heart dissected in situ after removal of the sternum and thymus. The pericardium has been removed. Note the relationship of the innominate vein to the aortic arch and the position of the arterial duct between the left pulmonary artery and the descending aortic arch.

Examination of the Heart

At postmortem, the heart should be examined in situ (Fig. 21.5). Its position in the chest and the position of its apex should be noted. The vascular connections should be examined. Particular care should be given to the venous system, noting the presence of the innominate vein once the thymus has been removed and whether there is a persistent left superior vena cava. The pulmonary venous connection should be established. This can usually be done by incising the pericardium and lifting up the apex of the heart. The left pulmonary venous attachments prevent the heart from being elevated far. The right pulmonary veins are more difficult to assess. The atrial appendages should be inspected to confirm situs solitus, and the relation of the great vessels noted in relation to each other. The relative size of the ventricles and of the atria can also be noted. The site of the aortic arch should be recorded, whether to the right or to the left. The branching pattern should be inspected, taking particular care to confirm the origin of the right subclavian artery from the brachiocephalic artery; if not, a retroesophageal right subclavian artery could be missed. The ductus should be inspected by folding forward the left lung, and the size of the aortic isthmus and juxtaductal area inspected; coarctation can very easily be missed if not specifically sought.

Ideally, and particularly where cardiac abnormality is known or suspected, the heart together with the lungs should be removed and fixed in formalin before further dissection. The tissues are easier to handle, and retain their relationships better when fixed and histological sampling is infinitely easier and more exact. This is not always possible, but even in cases where, for whatever reason, the heart cannot be retained, overnight fixation in 20% formalin will fix it sufficiently well for detailed examination and histological sampling.

Following fixation, the soft tissues should be dissected from around the heart and lungs. This is tedious but it does permit exact relationships to be inspected and recorded. Photographic records are very useful. Once the lungs have been detached the detailed analysis of the heart can begin.

The method of dissection self-evidently depends on the nature of the abnormality to be demonstrated, but, to some extent, methods are arbitrary and depend as much on personal preference as on scientific validation. My own preference in the uncomplicated case is to adopt the traditional method of following the flow of blood. The right atrium is opened by cutting from the inferior vena cava to the tip of the right atrial appendage. It is best not to open the superior vena cava. The superior caval junction with the right atrial appendages marks the site of the sinoatrial node, and cutting this area risks loss of landmarks should it become necessary to examine the node. Opening from the inferior vena cava displays all the right atrial structures; there is a good view of the atrial septum, and the appendage and coronary sinus can easily be inspected. The tricuspid valve can be inspected from above before cutting down the lateral border of the right ventricle through the atrioventricular junction to the apex. A further cut from the apex to the pulmonary artery completes the opening of the right heart. This method does destroy the continuity of the valves but allows close inspection of their constituent parts. The right ventricular aspect of the interventricular septum is exposed, as is the pulmonary infundibulum. The incision may be extended through the ductus arteriosus into the aorta. Alternatively, the ductus may be probed and sectioned transversely for histological examination.

The left atrium is opened between the pulmonary veins. The orifices of the pulmonary veins should be probed, because even in pulmonary vein stenosis, the vessels may appear to be of good caliber externally. The left ventricle is opened by cutting down the free wall of the ventricle through the atrioventricular junction to the apex. To expose the outflow tract, one of two approaches may be used, neither of which is without disadvantages. The aortic outflow can be exposed by cutting up through the aortic leaflet of the mitral valve. This destroys the integrity of the valve but exposes the coronary arteries well and does not transect the proximal left coronary artery. Alternatively, the incision may be made through the anterior wall of the ventricle close to the septum, leaving the anterior leaflet of the mitral valve intact but transecting the left coronary artery near its origin. The incision is extended along the length of the aortic arch on its convex aspect to permit inspection of the junction of the ductus and aorta. The coronary artery ostia should be inspected carefully and the arteries may be probed with a very fine probe. Their course on the epicardium should be noted and the artery supplying the posterior interventricular artery identified.

If no macroscopic abnormality is seen, adequate sampling for histology includes right and left atrioventricular junctions with papillary muscles, a transverse section through the midpoint of the interventricular septum to include left and right free wall attachments and a vertical block to include the membranous septum and atrioventricular conduction axis.

This approach works well in the neonatal and older heart, although marked ventricular hypertrophy can make internal inspection difficult. For smaller hearts and particularly for small, abnormal macerated hearts, formalin fixation of the heart is essential followed by examination with some form of magnification system. I prefer a dissecting microscope because of the clarity of detail and the range of magnification available. For embryos, especially from ruptured ectopic pregnancies, embedding the embryo whole and serial sectioning is the only sure way of thorough examination.

Weights and Measures

Standard tables are available giving ranges for normal hearts throughout gestation and in infancy and childhood. As a minimum, the heart weight should be recorded (Rowlatt et al. 1963; Eckner et al. 1969).

Structural Congenital Heart Disease

Although the term *congenital heart disease* is used synonymously with structural congenital heart

Table 21.1. Common structural congenital heart defects in decreasing order of frequency

Ventricular septal defect
Patent arterial duct
Atrial septal defect
Tetralogy of Fallot
Pulmonary stenosis
Coarctation of the aorta
Aortic stenosis
Transposition of the great arteries

disease, it should be borne in mind that other forms of heart disease, lacking structural defects, can also develop in utero and be present at birth, for example, cardiomyopathy, rhythm disorders, and myocardial infarction.

There are eight common lesions that together account for about 80% of all cases of structural congenital heart disease (Jordan and Scott 1989). They are listed in Table 21.1.

Ventricular Septal Defect

A ventricular septal defect (VSD) forms an integral part of many complex abnormalities of cardiac structure, but may occur in isolation. As the name implies, it is a communication between the right and left ventricular cavities. Its clinical effect depends on its size and the presence or absence of associated lesions. The defects are usually round or oval and vary in size in the neonate from a few millimeters to 1.5 cm (Arey 1984a).

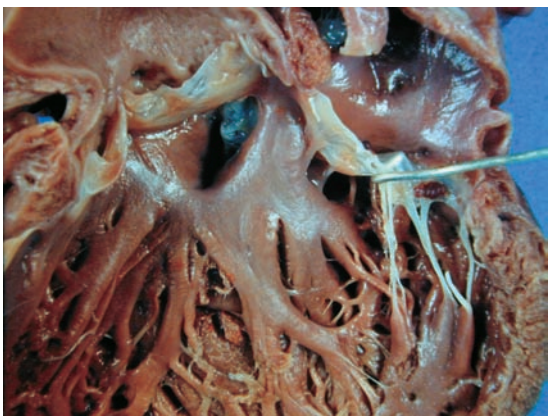

Figure 21.6. Perimembranous ventricular septal defect. The left ventricular outflow tract has been opened and the anterior leaflet of the mitral valve drawn aside to reveal a triangular ventricular septal defect lying beneath the right coronary and noncoronary cusps of the aortic valve, a position normally occupied by the membranous interventricular septum.

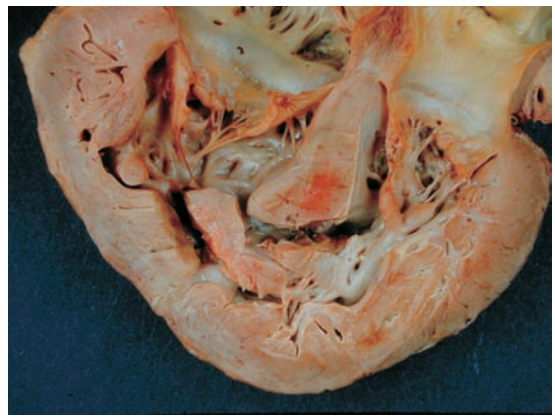

Figure 21.7. Muscular ventricular septal defects. The heart has been cut in a simulated four-chamber view. The apex of the interventricular septum shows several tortuous channels connecting the right and left ventricles. They are lined by opaque and thickened endocardium. There is hypertrophy of the right ventricular myocardium. Muscular ventricular septal defect (VSD) can be very hard to identify, being obscured by hypertrophied ventricular trabeculations.

Two basic forms are described depending on their relation to the membranous interventricular septum: those involving the membranous septum, the so-called perimembranous VSD, and those in which muscle is interposed between the defect and the membranous septum, the so-called muscular VSD. Because the tricuspid valve attachment crosses the membranous septum and the mitral valve attachment on the left side is also related to the membranous septum, a perimembranous defect, by definition, has fibrous continuity between the tricuspid and mitral valves through the defect (Fig. 21.6). Muscular VSDs may occur at any part of the interventricular septum and may be multiple (Fig. 21.7). Because of associated ventricular hypertrophy, those occurring in the lower parts of the septum can be easily missed, even on close inspection of the heart, being hidden among the hypertrophied muscular trabeculations. Perimembranous and muscular VSD can, and often do, coexist in the same heart.

The significance of the distinction of perimembranous and muscular VSD lies in the intimate and superficial relation of the atrioventricular conduction tissue to the perimembranous VSD and its susceptibility to damage during surgical closure of the defect. The VSDs may lie in

the inlet, outlet, or apical trabecular part of the ventricular septum. They may also be closely related to the arterial valves. In the setting of a double outlet ventricle, the outflow from the smaller ventricle is dependent on the size of the VSD and, if too small, the VSD is said to be restrictive. Small perimembranous VSDs are usually better appreciated from the left ventricular outflow tract; on the right side they may be obscured by the chordal and leaflet tissue of the tricuspid valve.

A VSD occurring in the right ventricular outflow tract may permit fibrous continuity between the aortic and pulmonary valves. Such defects (which may be either muscular or perimembranous) are said to be doubly committed and juxtaarterial (Anderson et al. 1984).

Unless there is restricted outflow through the pulmonary valve, blood tends to flow through the VSD from left to right ventricle because of the pressure gradient between the two, and the right ventricle suffers volume overload. Ventricular septal defects, if small, may close spontaneously by fibrosis (Alpert et al. 1979), but tissue tags associated with VSD may cause subvalvular obstruction. The VSD margins are a site of predilection for infective endocarditis.

Atrioventricular Septal Defect

The basic abnormality in this defect is a common atrioventricular junction, in contrast to the separate right and left atrioventricular junctions present in the normal heart (Becker and Anderson 1982). The junction is guarded by a common valve (Fig. 21.8) and, because of the common junction, the aorta is displaced from its normal position (where it is wedged between the separate left and right atrioventricular junctions) to lie anterior to the common atrioventricular junction. The inferior aspect of the interatrial septum is not connected to the ventricular septum and is a free-standing structure; the upper border of the interventricular septum has a scooped-out appearance. The common valve has five leaflets: superior and inferior bridging leaflets that cross (bridge) the interventricular septum and that have papillary muscle attachments in both ventricles; a mural leaflet on the left side that is smaller than its normally occurring counterpart, and anterior and inferior leaflets on the right. The valvular tissue

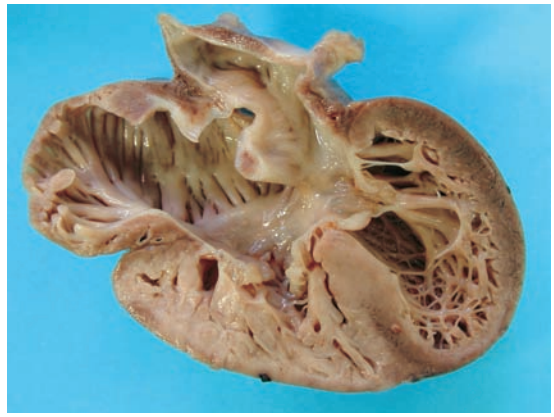

Figure 21.8. Atrioventricular septal defect. The heart is cut in a simulated four-chamber view. There is a large defect between the lower border of the interatrial septum and the crest of the muscular interventricular septum. An inferior bridging leaflet crosses the inferior aspect of the defect and shows some tethering to the crest of the septum. There is also a defect in the oval fossa. The right atrium is grossly dilated.

may be dysplastic and valvular regurgitation is common.

Variations in the attachment of the bridging leaflets give rise to the various subgroups of this defect (Rastelli et al. 1966). Where the bridging leaflets float freely and are attached to neither interatrial nor interventricular septum, nor to each other the defect is a complete atrioventricular septal defect (AVSD). There is free communication of blood between both ventricles beneath the leaflets and between both atria above them. Where there is attachment of the bridging leaflet tissue to the crest of the interventricular septum or where a tongue of leaflet tissue joins the two leaflets over the interventricular septum, there is some restriction in the mixing of blood at the ventricular level and the defect is called a partial AVSD. Where the bridging leaflets are attached to the crest of the interventricular septum so as to obliterate the interventricular communication, the defect becomes, in effect, an interatrial communication and is the so-called ostium primum atrial septal defect. That the ostium primum defect is, in reality, an atrioventricular septal defect is reflected in the features it shares with other forms of AVSD: the common atrioventricular (AV) junction (despite separate orifices), the unwedged aorta, the scooped out interventricular septal crest, and the vestige of the fused bridging

leaflets in the misnamed "cleft" anterior leaflet of the mitral valve.

In an AVSD, the relative sizes of both ventricles can vary and there is often disproportion (Anderson et al. 1998). An AVSD can coexist with other forms of congenital heart disease such as arterial valve obstruction. It is an almost universal finding in cases of right atrial isomerism. An AVSD is also the characteristic cardiac defect occurring with trisomy 21. Because of anterior displacement of the aorta in this defect, the aortic outflow tract is lengthened and narrowed. While of itself not causing significant obstruction, it takes little additional obstruction, for example by valvular tissue tags, to cause clinical symptoms. Because the structures of the membranous septum are absent, the atrioventricular conduction tissue is abnormally sited. The atrioventricular node, instead of being located in the usual site of the triangle of Koch, is located more inferiorly in the so-called nodal triangle whose borders are the atrioventricular junction, the mouth of the coronary sinus, and the posteroinferior leading edge of the atrial septum (Thiene et al. 1981). The bundle of His is unusually long and travels on the crest of the interventricular septum before dividing.

Presentation is in the first few weeks rather than the first days of life and with cardiac failure. Cyanosis is usually not evident.

The defect is treated by early operation with a two-patch repair (Weintraub et al. 1990). In untreated cases, unless there is associated pulmonary stenosis, pulmonary hypertension develops rapidly and plexiform lesions may be evident in the lungs within the first year of life.

Atrial Septal Defect

The majority of atrial septal defects (ASDs) occur within the oval fossa. Such defects are, by definition, of secundum type. Conversely, any atrial septal defect occurring outside of the oval fossa cannot be of secundum type. In about 10% to 20% of the general population there is probe patency of the interatrial septum at the anterosuperior part of the oval fossa (Hagen et al. 1984; Fisher et al. 1995). It is sometimes referred to as persistent foramen ovale (PFO). Because under normal conditions the pressure of blood in the left atrium is higher than in the right, the flap remains closed. Deficiency of the anterosuperior aspect of the flap valve of the oval fossa is responsible for true secundum atrial septal defects (Fig. 21.9). The

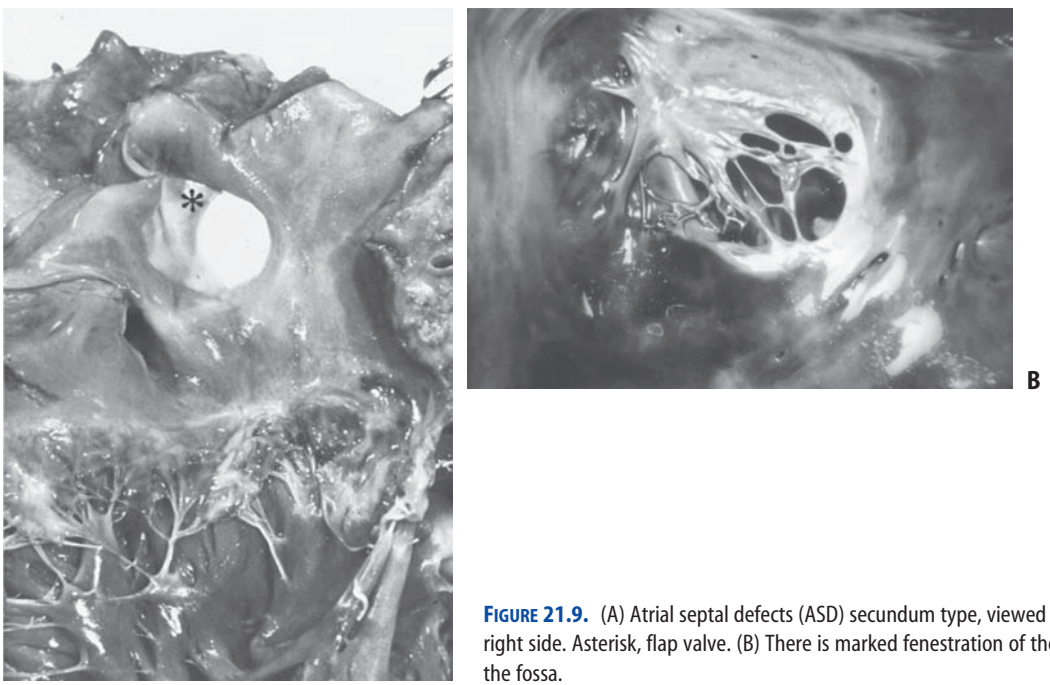

Figure 21.9. (A) Atrial septal defects (ASD) secundum type, viewed from the right side. Asterisk, flap valve. (B) There is marked fenestration of the floor of the fossa.

defects may involve, when extreme, the entire oval fossa or merely a tiny part. The defect may be fenestrated with multiple small holes within the flap valve. There may be slight surrounding endocardial fibroelastosis but usually no other consequence. An ASD may occur in combination with any other cardiac defect and in some cases is essential for the continued well-being of the child, for example, in complete transposition of the great arteries with intact ventricular septum. When an atrial septal defect is not present, one has to be created artificially to permit mixing of both pulmonary and systemic circulations and ensure survival.

Other types of ASD, with the exception of the ostium primum defect, which in reality is a form of AVSD (discussed above), are very rare. They are the coronary sinus defect (Lee and Sade 1979) and the so-called sinus venosus defect (Al Zaghal et al. 1997), which is usually associated with anomalous drainage of the right pulmonary veins through the defect into the right atrium.

Abnormalities of the Arterial Duct (Ductus Arteriosus)

The arterial duct is a muscular artery interposed between the two elastic arteries of the aorta and pulmonary trunk (Silver et al. 1981). It runs from the posterosuperior aspect of the junction of the pulmonary trunk with the left pulmonary artery, upward and slightly laterally, and inserts into the medial aspect of the aorta just distal to and opposite the left subclavian artery. It develops from the embryonic sixth aortic arch.

Variations in anatomy occur normally; some ducts are long and others short. There is also some variation in the angle of entry of the duct into the aorta, and some authors consider this of importance in ductal closure (Rudolph 2001a). In the presence of a right-sided aortic arch, the duct usually remains on the left side, taking origin from the left subclavian artery or the innominate artery.

Absence of the Arterial Duct

In tetralogy of Fallot there is absence of the duct in approximately one fifth of cases. Truncus arteriosus usually lacks an arterial duct and cases of pulmonary atresia with VSD and major aortopulmonary collateral arteries also lack an arterial duct. Bilateral arterial ducts may also occur in some forms of interrupted aortic arch or with isolated left pulmonary artery.

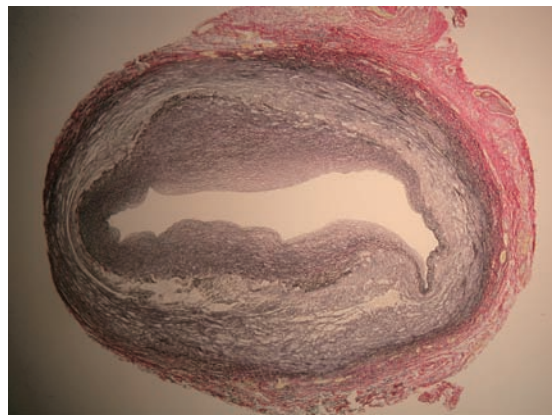

Figure 21.10. Arterial duct at term. Elastic van Gieson preparation of a transverse section through the central part of the duct. The tunica media shows lucent areas occupied by myxoid material, and the intima shows irregular thickening by fine elastic fibers with discontinuities in the underlying internal elastic lamina.

Normal Closure

Having, in the normal course of events, become redundant at birth, the arterial duct closes by muscular contraction in the first 24 hours; this is manifest as shortening and thickening of the duct. This approximates the intimal cushions that form in late fetal life, enhancing the closure, and causes ischemia of the muscle of the duct leading to fibrosis of the media (Fig. 21.10). Over the following weeks permanent closure is achieved by mural fibrosis, and the duct is converted into a thick fibrous cord—the ligamentum arteriosum—that frequently calcifies (Silver et al. 1981). Closure is usually complete by 3 to 4 weeks of age.

Premature Closure In Utero

This is rare and may be associated with in-utero right heart failure, fetal distress, hydrops, or intrauterine death. The duct is contracted and may still be probe patent. The presence of thrombus assists greatly in making the diagnosis. Maternal ingestion of nonsteroidal antiinflammatory drugs is reported in some cases (Schiessl et al. 2005).

Persistent Patency of the Duct

The duct remains functionally patent for the first few days of life in most premature infants (Tynan 1993). Administration of indomethacin, an inhibitor of prostaglandin synthesis, causes closure of the duct in most, but not all, cases, and a closed duct may reopen by poorly understood mechanisms (Silver et al. 1981). There is evidence that persistent patency of the duct is associated with the development of chronic lung disease in premature infants (Bancalari 2001).

Persistent patency of the duct occurs in association with various forms of structural heart disease, its patency in some being essential to life. Patent duct and peripheral pulmonary artery stenosis are the commonest cardiac manifestation of maternal rubella infection.

A persistently patent arterial duct is at risk of development of infective endocarditis.

Aneurysm of the Ductus

This is a rare occurrence. Cases may be associated with lamina thrombus and tears in the intima, or with infection (Lund et al. 1992) (Fig. 21.11).

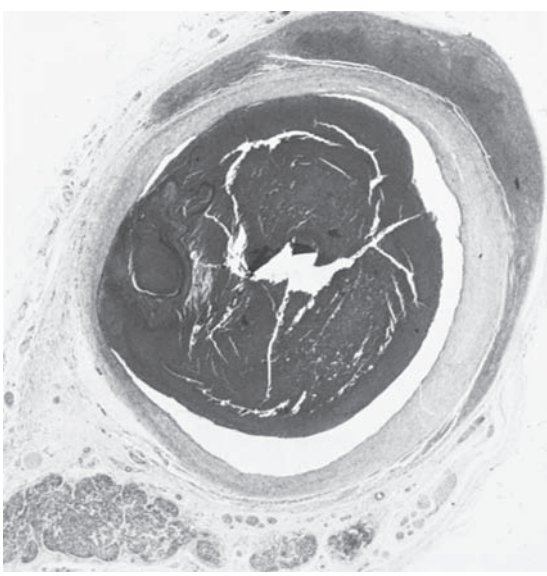

Figure 21.11. Aneurysmal dilatation and thrombosis of the ductus arteriosus with mural hemorrhage suggestive of dissection.

Coarctation of the Aorta

Coarctation is a narrowing of the aorta at the site of insertion of the arterial duct. The narrowing may be discrete or it may be accompanied by tubular hypoplasia of the aorta (Pellegrino et al. 1985). The discrete lesion is usually evident externally as a notch in the aortic arch wall, most prominently on its convex aspect opposite the ductal insertion; internally there is a shelf of tissue protruding into the lumen from the side of aortic wall opposite the site of insertion of the arterial duct (Fig. 21.12A). A degree of poststenotic dilatation may be visible, but in infants it is usually not marked. The normal aortic isthmus (that segment of the aortic arch between the left subclavian artery and the arterial duct) is normally narrower than the remainder of the aorta, and it is important not to mistake this normal appearance for tubular hypoplasia.

Coarctation of the aorta may occur as an isolated lesion, or it may accompany other cardiovascular malformations, most notably ventricular septal defect and left-sided obstructive lesions (Becker et al. 1970).

Histologically, a sling of ductal tissue extends around the aortic wall causing the narrowing (Fig. 21.12B). Secondary intimal fibrous proliferation further narrows the lumen (Elzenga and Gittenberger de Groot 1983). Following resection of the affected segment, recoarctation may occur if sufficient ductal tissue is left in the aortic wall (Russell et al. 1991).

Those infants with severe coarctation present in the first weeks of life with evidence of cardiac failure.

Pulmonary Atresia and Stenosis Including Tetralogy of Fallot

Congenital atresia of the pulmonary valve may occur with an intact interventricular septum or may be coupled with a ventricular septal defect. The two entities are distinct clinically and have distinct pathological associations. There may be severe stenosis of the pulmonary valve instead of atresia, and, indeed, there is evidence from serial ultrasound scanning in utero that some cases of pulmonary atresia develop from pulmonary stenosis.

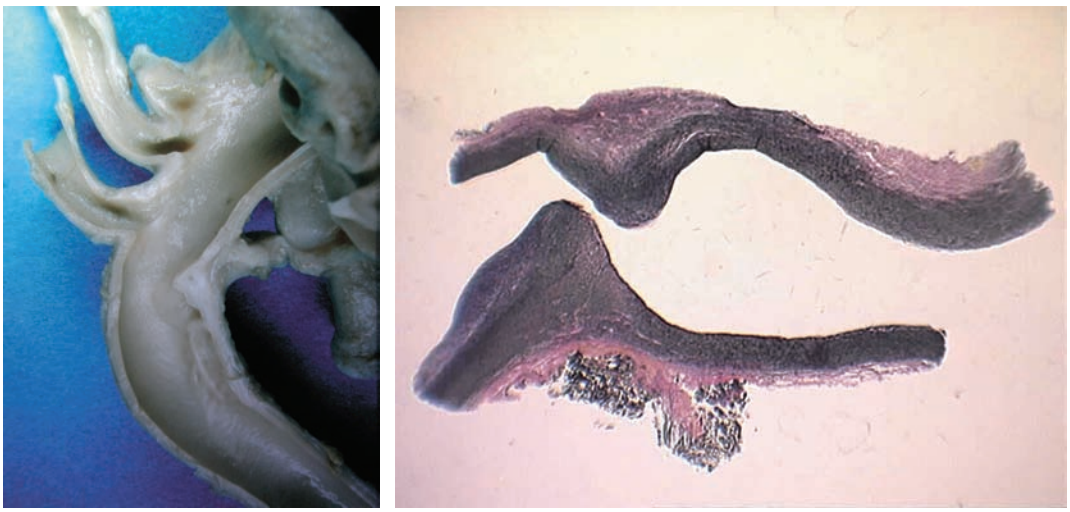

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Figure 21.12. (A) Coarctation of the aorta. A longitudinal section of the aortic arch and descending aorta viewed from behind. Just distal to the origin of the left subclavian artery and the point of insertion of the arterial duct a triangular shelf of white tissue protrudes into the aortic lumen causing narrowing. (B) Coarctation of the aorta. Elastic van Gieson stained longitudinal section through the point of coarctation showing an hourglass constriction of the lumen caused by pale-staining ductal tissue in the tunica media. There is further narrowing of the lumen by secondary intimal elastic thickening.

Pulmonary Atresia with Intact Interventricular Septum

This lesion usually occurs as part of more complex malformations. As an isolated lesion it is uncommon (1% to 4% cases of congenital heart disease) (Daubeney et al. 2002). It almost always occurs with concordant atrioventricular and ventriculoarterial connections. Pathologically it is characterized by patency of the oval foramen and of the arterial duct. The right atrium is dilated, the degree of dilatation being related to the extent of the right ventricular dilatation. The right ventricle is usually small with a hypertrophied wall but, in those cases in which the tricuspid valve is not competent, the right ventricle may be of normal size. The degree of ventricular hypertrophy may be so great as to obliterate the outlet and apical trabecular components of the right ventricle, leaving only an inlet component (Fig. 21.13). The tricuspid valve is small, and there is associated Ebstein's malformation in about 10% of cases. The pulmonary trunk is small but may be of normal size (it is rarely atretic), and the valve

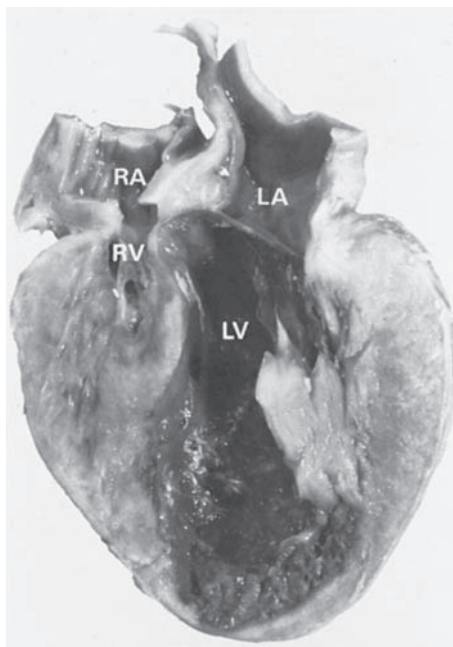

Figure 21.13. Pulmonary valve atresia with tricuspid valve stenosis. There is a right-sided rudimentary ventricle. RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle.

annulus is narrow. The pulmonary valve is imperforate, being convex toward the pulmonary trunk and showing three ridges radiating from a central fibrous button. The branch pulmonary arteries are thin-walled. The left ventricle is also hypertrophied. The ascending aorta is wide and the normal narrowing of the isthmus is absent. The arterial duct is narrow and arises from the descending aorta with an acute inferior angle.

A notable feature is the presence of right ventricular–coronary artery sinusoids. These are said to arise from persistence of the normal ventricular–coronary communications in the embryo because of persistently elevated right ventricular pressure. However, there is one report of identification of ventricular coronary artery sinusoids before the onset of pulmonary obstruction (Gittenberger de Groot et al. 2004). The sinusoids are readily demonstrable on echocardiography but are very difficult to demonstrate convincingly pathologically. They do, however, cause dramatic secondary changes in the affected coronary arteries, which show greatly thickened muscular walls, intimal fibroelastic thickening, and adventitial elastic deposition (Fig. 21.14). Sometimes the epicardial coronary arteries may be two to three times their normal size. On occasion the affected coronary artery loses its communication with the aorta because of the intimal proliferation, and the perfusion of the myocardium supplied by that artery may be critically dependent on the elevated right ventricular pressure for retrograde perfusion. The hypertrophied right ventricular myocardium shows myofiber disarray.

Pulmonary Stenosis with Intact Interventricular Septum

Stenosis of the pulmonary valve is less common in the neonate than atresia. The morphology of the valve may be reminiscent of that in atresia but with a central lumen (Stamm et al. 1998), or may comprise three cusps that are thickened and dysplastic (Fig. 21.15). The severity of the stenosis depends on the pressure gradient across the right ventricular outflow tract; gradients of between 40 and 80 mm Hg are considered moderate and above 80 mm Hg are considered severe. Experimentally, the pulmonary artery must be constricted to one third of its diameter to produce a significant pressure gradient. The right ventricle is hypertrophied, as is the right atrium because of the increased diastolic filling pressure. The pulmonary trunk is large and thin-walled because of poststenotic dilatation. Some cases show a thickened tricuspid valve and jet lesions in the right atrium. Pulmonary stenosis may occur in Williams syndrome or Noonan syndrome (Sreeram et al. 1994) or as a result of maternal rubella infection.

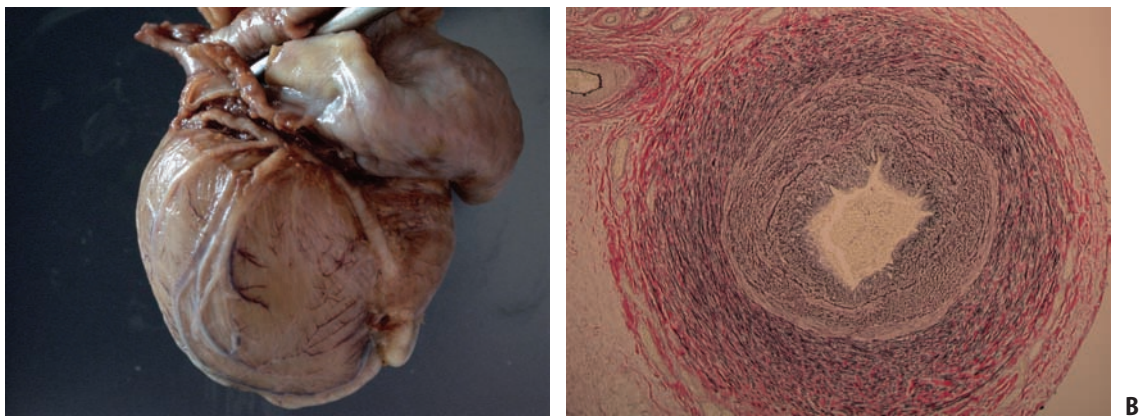

Figure 21.14. (A) Pulmonary atresia with intact septum. External view of the inferior surface of the heart showing marked thickening of the epicardial coronary arteries most noticeable on the right hand edge of the specimen where the artery is white and tortuous and protrudes from the epicardium. (B) Microscopic view of the same vessel cut in transverse section and stained with elastic van Gieson. The adventitia shows thickening by concentric fibroelastic tissue, there is elastic deposition in the media and the intima also shows fibroelastic irregular thickening.

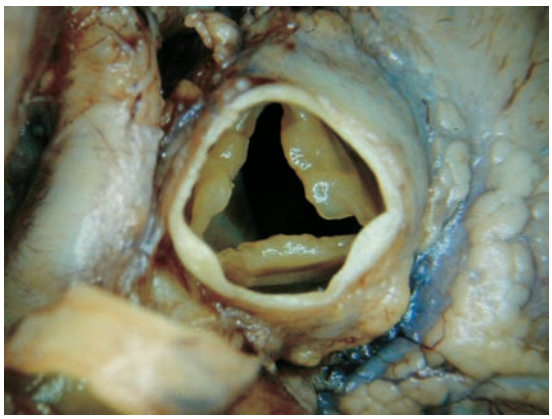

Figure 21.15. Stenotic, dysplastic pulmonary valve viewed from above. The edges of the leaflets are rolled, myxoid, and thickened.

Pulmonary Stenosis with Ventricular Septal Defect, Including Tetralogy of Fallot

Tetralogy of Fallot refers to a group of four abnormalities occurring together: pulmonary stenosis, VSD, overriding aorta, and right ventricular hypertrophy (Anderson et al. 1981a). The basic defect is anterior insertion of the ventriculoinfundibular fold into the anterior limb of the septomarginal trabeculation (normally the ventriculoinfundibular fold inserts between the limbs of the septomarginal trabeculation) (Fig. 21.16). This leaves a VSD posteriorly and causes narrowing of the right ventricular outflow tract. The aorta overrides the VSD and the presence of pulmonary stenosis leads eventually to right ventricular hypertrophy. About one quarter of cases show a right-sided aortic arch, and there may be absence of the arterial duct. The aorta is usually large and the pulmonary artery small, but they may be of equal size. The pulmonary trunk and pulmonary arteries are thin walled. The VSD is usually large and may extend to the membranous septum to become a perimembranous VSD. The degree of aortic override is also variable. If the degree of override is greater than 50%, it is arbitrarily classified as double outlet right ventricle. While infundibular stenosis is present in all cases, there is variable valve stenosis, and approximately 20% of cases have pulmonary atresia. The valve may have one, two, three, or even four cusps. The valve may be completely

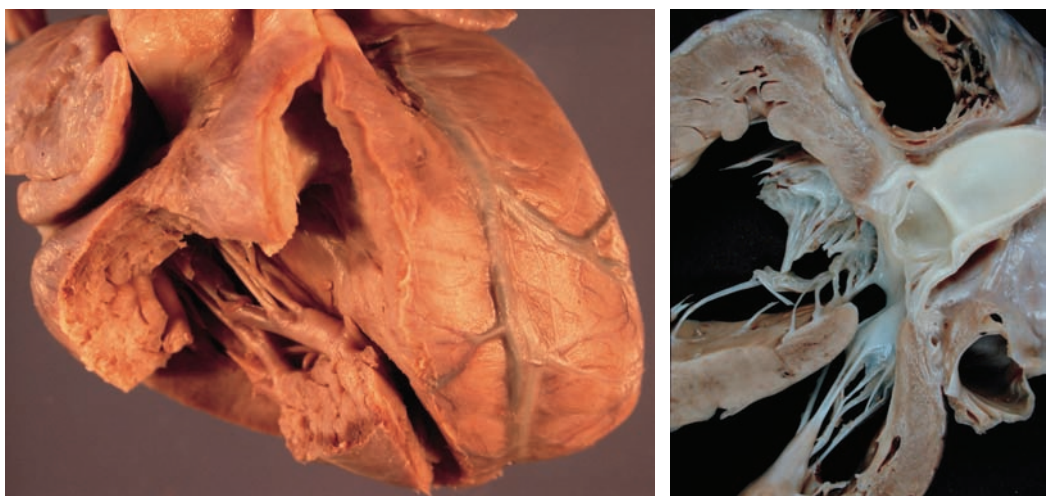

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Figure 21.16. (A) Tetralogy of Fallot. The hypertrophied right ventricular wall has been dissected away to demonstrate the muscular subpulmonary narrowing. The ventricular septal defect is just visible as a dark area lying to the left of the subpulmonary muscular bar. It can be seen that the aorta overrides this area. (B) Tetralogy of Fallot. Heart cut in a simulated five-chamber view to show the aorta overlying the right ventricle with a ventricular septal defect lying to the leftward side of the aorta.

absent with associated aneurysmal dilatation of the pulmonary trunk and absence of the arterial duct (Emmanouilides et al. 1976). There may also be supravalvular stenosis.

It must be emphasized that not all cases of pulmonary stenosis with VSD represent tetralogy of Fallot; only those (majority) of cases exhibiting the characteristic morphology of anomalous anterior insertion of the ventriculoinfundibular fold can be so designated.

Because of the reduction in pulmonary blood flow and shunting of blood from the right ventricle to the aorta, tetralogy of Fallot presents with cyanosis usually in the first few months of life. Sudden death may occur, even in operated cases (Zhao et al. 1985).

Pulmonary Atresia with Ventricular Septal Defect

This is a quite distinct abnormality from pulmonary atresia with intact septum. This lesion does not show the diminutive and hypertrophied ventricle of pulmonary atresia with intact septum. The pulmonary trunk is usually atretic, being represented only by a thin thread-like cord. The branch pulmonary arteries may be of normal size and supplied by an arterial duct. The duct may be absent, in which case the lungs derive their blood supply from major aortopulmonary collateral arteries (MAPCAs) (Liao et al. 1985). These vessels, some of which are hypertrophied bronchial arteries, arise from the aortic arch and descending aorta (Fig. 21.17). Many cases represent tetralogy of Fallot with pulmonary valvar atresia.

Absence of the Pulmonary Valve

In this condition, there is complete absence of the cusps of the pulmonary valve, or valvar tissue is represented only by rudimentary excrescences at the site of the valve. It is usually associated with a VSD and may occur in tetralogy of Fallot.

Characteristically, there is dilatation of the pulmonary artery, sometimes to aneurysmal proportions, and the vessel wall may be disorganized and calcified.

Aortic Stenosis

Typically aortic stenosis is classified into three forms: subvalvar stenosis, valvar stenosis, and supravalvar stenosis (Edwards 1965). Valvar stenosis is the commonest form.

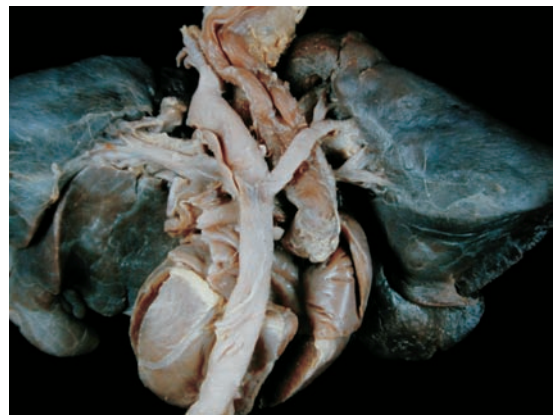


Figure 21.17. Pulmonary atresia with VSD. Specimen viewed from behind to demonstrate enlarged systemic arteries arising from the descending aorta to supply the lungs (major aortopulmonary collateral arteries).

Valvar Stenosis

Valvar stenosis may occur as an isolated lesion or may be associated with other abnormalities such as mitral stenosis, aortic coarctation, or VSD. The valve orifice is usually narrow, and there is fusion and dysplasia of the valve leaflet (McKay et al. 1992) (Fig. 21.18A). A dysplastic valve may be associated with dysplastic pulmonary or other valves in the so-called polyvalvar dysplasia (Bartram et al. 2001).

Subvalvar Stenosis

Subvalvar stenosis may be caused by muscular obstruction, as, for example in hypertrophic cardiomyopathy or by hypertrophy of an anomalous muscle bar or hypertrophied outlet septum in cases of double outlet ventricle. The obstruction may be caused by a subvalvar fibrous shelf (Fig. 21.18B) or may be caused by fibrous tissue tags or atrioventricular valvar tissue associated with a VSD.

Supravalvar Stenosis

Supravalvar aortic stenosis is caused by irregular thickening of the aortic wall some 1 to 2 cm above the aortic valve (Peterson et al. 1965). It is usually

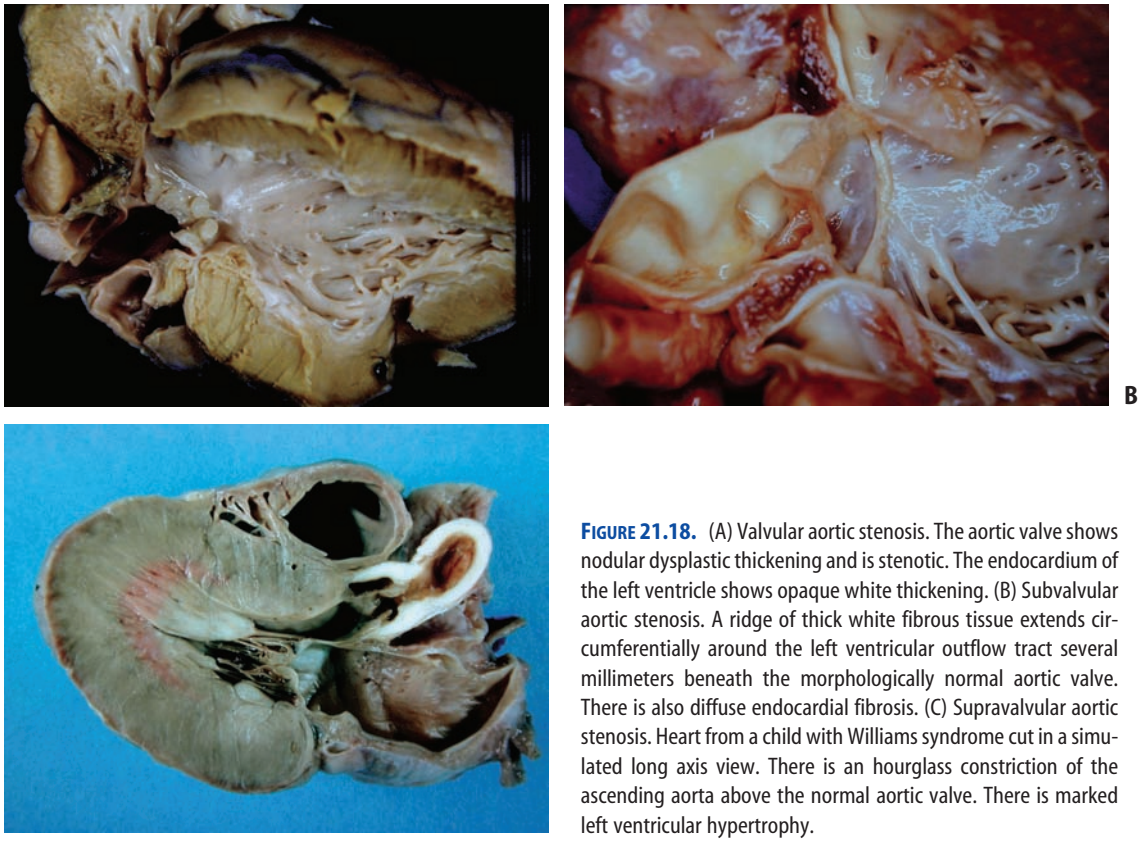


Figure 21.18. (A) Valvular aortic stenosis. The aortic valve shows nodular dysplastic thickening and is stenotic. The endocardium of the left ventricle shows opaque white thickening. (B) Subvalvular aortic stenosis. A ridge of thick white fibrous tissue extends circumferentially around the left ventricular outflow tract several millimeters beneath the morphologically normal aortic valve. There is also diffuse endocardial fibrosis. (C) Supravalvular aortic stenosis. Heart from a child with Williams syndrome cut in a simulated long axis view. There is an hourglass constriction of the ascending aorta above the normal aortic valve. There is marked left ventricular hypertrophy.

associated with Williams syndrome caused by mutations in the elastin gene on chromosome 7. The stenosis has a characteristic hourglass appearance when viewed externally (Fig. 21.18C). There is usually thickening and fibrosis of the coronary arteries (Van Son et al. 1994), the arteries bearing a striking resemblance to those seen in pulmonary atresia with intact septum and ventriculocoronary artery communications. The left ventricle is hypertrophied.

Hypoplastic Left Heart

This is a group of abnormalities characterized by a small left ventricle unable to support the systemic circulation (Salmon 2001). The usual case has a thread-like ascending aorta that is patent and supplies blood to the coronary arteries in a retrograde fashion. The mitral valve is usually small and dysplastic, in which case the ventricle shows marked endocardial fibroelastosis; if the mitral valve is atretic, there is no endocardial fibroelastosis. The left atrium is small. The aortic valve is usually atretic but may be severely stenotic and dysplastic, in which case the ascending aorta is correspondingly larger. The interventricular septum is usually intact but there may be a VSD if there is mitral atresia. Externally, the coronary arteries delimit the hypoplastic left ventricle. The left ventricular myocardium is hypertrophic, and 80% of cases show myofiber disarray histologically. The appearances are analogous to the right heart in pulmonary atresia with intact septum, and in some cases ventriculocoronary artery communications can also be demonstrated (Sauer et al. 1989; Connor et al. 1992). About two thirds of cases show coarctation of the aorta (Elzenga and Gittenberger de Groot 1985).

Transposition of the Great Arteries

In transposition there is ventriculoarterial discordance, the aorta arising from the right ventricle and the pulmonary artery from the left. In the

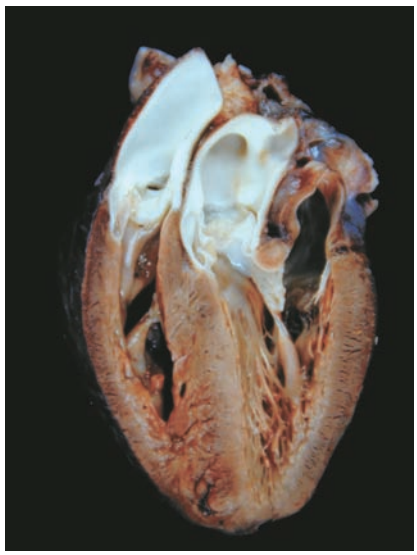


Figure 21.19. Transposition of the great vessels. Heart cut in a simulated long-axis view. The aorta arises from the right ventricle and the pulmonary artery from the left. The great arteries have a parallel configuration in contrast to the normal spiral arrangement. The ventricular septum is intact.

usual situation (complete transposition) there is atrioventricular concordance. If there is atrioventricular discordance the term *congenitally corrected transposition* is employed (Allwork et al. 1976). In the majority of cases the aorta is anterior and to the right of the pulmonary artery (Fig. 21.19). The aorta has a complete muscular infundibulum and the pulmonary valve is in fibrous continuity with the mitral valve. The aorta and pulmonary trunk lack the usual spiral relation to each other. Instead they arise parallel within the pericardial sac. Coronary artery origins from the aorta are more variable than usual (Yacoub and Radley Smith 1978). There may be an atrial septal defect, which allows some mixing of systemic and pulmonary circulations. When ASD is absent, a communication must be created artificially to permit survival until an arterial switch operation can be performed. Complete transposition may be complicated by VSD (approximately 20% of cases), coarctation (approximately 7% cases), or pulmonary stenosis (approximately 7% cases) (Anderson et al. 1991). In the absence of a VSD the baby appears normal at birth but becomes cyanosed on the first day of life and quickly becomes acidotic. In those cases with VSD, breathlessness and cardiac failure develop within the first week of life; cyanosis is minimal.

Treatment of complete transposition is neonatal arterial switch, where the pulmonary artery and the aorta are transected and switched to their correct ventricles. The coronary arteries are also switched, each with an attached button of surrounding arterial wall. This is the most critical aspect of the operation.

The term *congenitally corrected transposition* refers to ventriculoarterial discordance in the setting of atrioventricular discordance (Allwork et al. 1976). The vast majority of these hearts have associated cardiac malformations with most having abnormalities of the tricuspid valve and a VSD.

Common Arterial Trunk (Truncus Arteriosus)

This lesion is uncommon, accounting for about only 0.5% of all cases of structural congenital heart disease. A single arterial trunk arises from the base of the heart that gives rise to the pulmo-

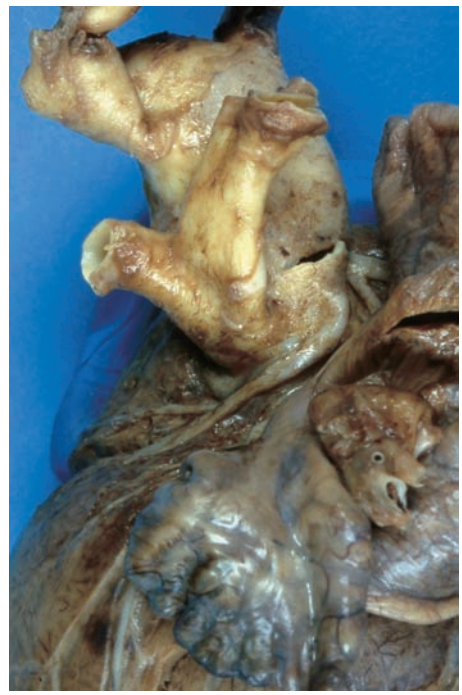


Figure 21.20. Common arterial trunk. The base of the heart is viewed from the left posterior aspect. A single great artery arises from the base and gives rise to the coronary arteries and the pulmonary trunk.

nary arteries, the systemic arteries, and the coronary arteries (Fig. 21.20). Self-evidently, there is a subarterial VSD. The truncal valve may have three, two, four, or even five leaflets and may be dysplastic. The origin of the pulmonary arteries from the trunk is the basis of the classification of this lesion (Collett and Edwards 1949). Type I shows a single pulmonary artery arising from the trunk, which then divides into right and left pulmonary arteries. In type II the pulmonary arteries arise separately but very close together from the trunk, and in type III the pulmonary arteries arise from the trunk but with widely separated orifices. The arterial duct is usually, but not invariably, absent. The coronary artery origins and course are abnormal (de la Cruz et al. 1990). If the truncal valve is competent, there are few or no symptoms in the first couple of weeks of life. Falling pulmonary vascular resistance with consequent increased pulmonary blood flow leads to the development of breathlessness and heart failure. If the valve is incompetent, symptoms occur in the first days of life.

Double Inlet Ventricle

This term describes an abnormal atrioventricular connection. Both atria are connected to one ventricle, either a morphologically right or morphologically left ventricle—the dominant ventricle. The inlet may be via two separate valves or via a common valve. The other ventricle is usually rudimentary and connected to the dominant ventricle via a VSD (Fig. 21.21). By definition, the rudimentary ventricle lacks a connection with the atria, but is connected to one of the great arteries. The term *univentricular heart* is sometimes applied to these cases, even though two ventricles, albeit one is rudimentary, are present; the heart is functionally univentricular.

Double Inlet Left Ventricle

This is the commoner of the two forms of double inlet ventricle (Anderson et al. 1984). The left ventricle is identified by the pattern of trabeculations on its septal surface. The rudimentary right ventricle is usually situated on the anterosuperior aspect of the ventricular mass and is delimited externally by the epicardial coronary arteries. In most cases, there is ventriculoarterial discordance, the pulmonary artery arising from the dominant left ventricle and the aorta from the rudimentary right ventricle. The VSD is usually restrictive and there is coarctation of the aorta. There may be subpulmonary stenosis. The term *Holmes heart* is applied if the ventriculoarterial connection is concordant. The conduction tissue is abnormally located.

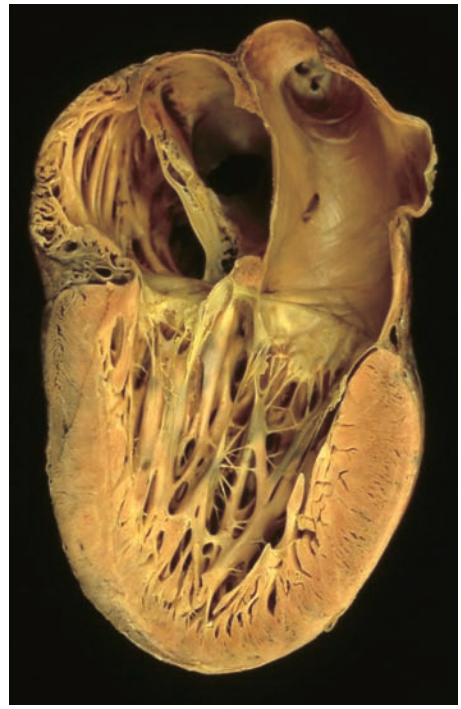


Figure 21.21. Double inlet left ventricle. In this section both atria enter the left ventricle but have separate orifices. Neither the great arteries nor the rudimentary right ventricle are visible in this section. The pulmonary artery arose from the left ventricle, and the rudimentary right ventricle was connected via a VSD to the left anterior aspect of the left ventricle and gave rise to the aorta.

Double Inlet Right Ventricle

This malformation is very rare and extreme forms may show double inlet and double outlet from the dominant right ventricle. The rudimentary left ventricle lies posteroinferiorly in the ventricular mass.

Double Outlet Ventricle

The double outlet ventricle is a ventriculoarterial connection in which both great arteries (or to be more precise more than half of each great artery)

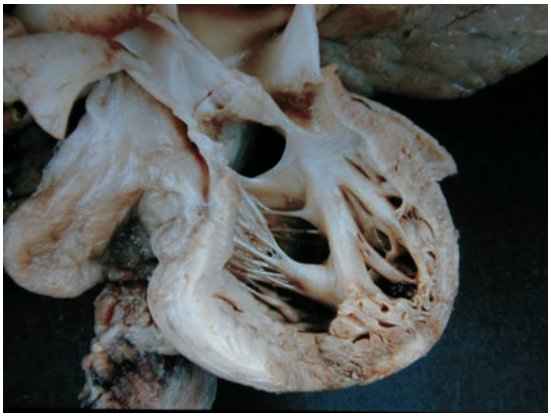


Figure 21.22. Double outlet right ventricle. Both great arteries arise from the right ventricle and there is a subaortic VSD. This specimen represents an extreme form of tetralogy of Fallot.

arise from the same ventricle (Anderson et al. 2001). Naturally, that ventricle may be either a right or a left ventricle, or even an indeterminate one. The term encompasses a host of conditions, some dominated by other features such as atrial isomerism.

Double Outlet Right Ventricle

Double outlet from the right is much commoner than double outlet from the left ventricle, although in overall terms both conditions are rare. In the usual setting of atrial situs solitus and concordant atrioventricular connection, double outlet right ventricle shows a ventricular septal defect, usually of perimembranous type, located between the limbs of the septomarginal trabeculation, beneath the outflow tract of the aortic valve. When both valves arise exclusively from the right ventricle, the aorta has a complete muscular infundibulum and the outlet septum is exclusively a right ventricular structure (Fig. 21.22). It shows anterior deviation to cause subpulmonary obstruction. If the aortic valve overrides the VSD, the morphology then resembles that of tetralogy of Fallot. A less common variant is where the VSD is subpulmonary, but still between the limbs of the septomarginal trabeculation—the so-called Taussig-Bing heart. There is usually associated subaortic stenosis or coarctation of the aorta.

Double outlet right ventricle is a common feature of hearts with right atrial isomerism. Pulmonary atresia or stenosis occurs in approximately half the cases.

Abnormalities of the Pulmonary Veins

Anomalous Pulmonary Venous Connection

This connection may be partial or total (DeLisle et al. 1976). Although the veins may connect separately to an anomalous site, it is usual for them to join to form a single channel that then connects anomalously. The anomalous connection may be to the heart itself or to the veins draining to the superior vena cava (supracardiac) or to those draining to the inferior vena cava (infracardiac) (Fig. 21.23). Frequently there is an element of obstruction to flow in the anomalous pathway. Anomalous venous connection may occur as an isolated abnormality, but may also occur as part of a more complex malformation. Morphologically, the left atrium is small in total anomalous pulmonary venous connection because of the lack

Figure 21.23. Total anomalous pulmonary venous connection. The organs are viewed from behind. The pulmonary veins come to a confluence behind the left atrium. Instead of being connected to the atrium, they form a descending vein that passes through the diaphragm to connect to the ductus venosus on the posterior aspect of the liver. As is usual in such cases, there is stenosis of the junction of the pulmonary venous trunk with the systemic venous system.

of pulmonary venous inflow. Pulmonary hypertension develops early. Clinically, the infant with nonobstructive total anomalous pulmonary venous connection does not present with symptoms for the first few months of life. Respiratory difficulty and failure to thrive are the commonest features. However, if obstruction is present, presentation is in the first few days of life with cyanosis and respiratory difficulty.

The sites of infracardiac connection include portal vein, hepatic vein, and ductus venosus. Connection is rarely directly to the inferior caval vein. Obstruction is much more likely with infracardiac connection.

Supracardiac connection is commoner and may be to the superior vena cava (on either side), azygos vein, or innominate vein. Cardiac connection is almost always to the coronary sinus.

Partial anomalous pulmonary venous connection may involve one or more veins and may involve the drainage from a whole lung. Partial anomalous connection is an integral part of the sinus venosus defect. It is also a component of the scimitar syndrome (Neill et al. 1960).

Pulmonary Vein Stenosis

Pulmonary vein stenosis usually affects all four pulmonary veins, but may be unilateral or affect a single vein (Sun et al. 1995). The vein is affected at its junction with the left atrium; the stenosis can be discrete or tubular. The narrowing may be visible externally, or the affected vessels may appear macroscopically normal from the outside (Fong 1988; Sun et al. 1995) (Fig. 21.24).

Obstruction to pulmonary venous return causes pulmonary hypertension; the veins show medial hypertrophy and fibrous intimal thickening, and the arteries show medial hypertrophy with or without intimal fibrous proliferation. The changes of pulmonary arterial hypertension may be present in both lungs even in the presence of unilateral stenosis.

Histologically, the stenotic segment of vein shows fibroelastic thickening of the intima (Fong 1988). There may be associated disruption and irregularity of the media. There may be associated intracardiac abnormalities. The stenosis is congenital and is thought to develop in utero after the incorporation of the pulmonary veins into the left atrium. The lesions may progress by the development of thrombus.

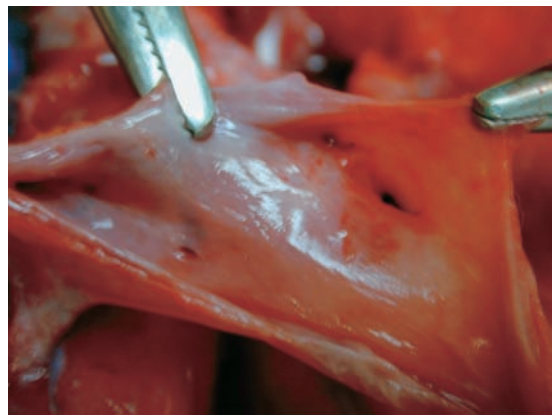


Figure 21.24. Pulmonary vein stenosis. The left atrium has been opened from the front to demonstrate the pin-hole orifices of the pulmonary veins at their junction with the left atrium. Externally, the veins appeared to be of normal caliber.

In its most severe form, congenital pulmonary vein stenosis is a progressive disease with rapid pulmonary hypertension and rare survival beyond the first year of life.

Ebstein's Malformation

The essential defect in this malformation is an abnormally low attachment of the tricuspid valve. The attachment, instead of being at the atrioventricular junction, is in the inlet part of the right ventricle (Anderson and Lie 1978). The septal and inferior leaflets show the abnormal attachment, and the septal leaflet is sometimes no more than a row of nodular excrescences descending in an oblique line on the right aspect of the interventricular septum. The anterosuperior leaflet is also abnormal, being large and rectangular and attached to the papillary muscles in such a way as to obstruct the inflow. The valve tissue is frequently dysplastic with redundant valvar tissue. The area of ventricular myocardium incorporated into the right atrium by the abnormally low attachment of the valve becomes thin and "atrialized." The valve is incompetent, and there is massive dilatation of the right atrium. Pulmonary stenosis (or atresia) is a frequent association. The condition may present in utero with cardiac failure and hydrops (Fig. 21.25).

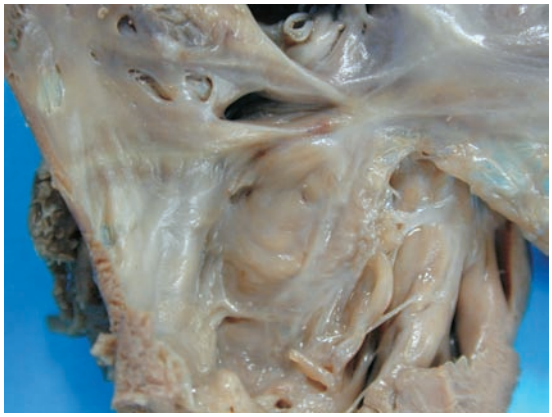


Figure 21.25. Ebstein's anomaly. The right atrioventricular junction has been opened. The junction runs horizontally across the picture and is visible as a pale line beneath the coronary sinus. The attachment of the septal leaflet of the tricuspid valve to the septum, instead of being at the atrioventricular junction, plunges downward on the septum toward the apex, leaving a large area of the right ventricle included in the atrium. The septal leaflet is malformed, comprising small nodular collections of myxoid tissue.

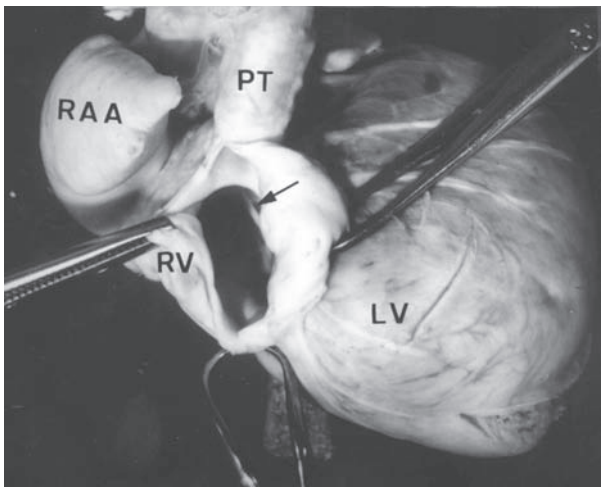
In trisomy 21, there may be a defect in the tricuspid valve with absence of the commissure between the anterosuperior and septal leaflets, sometimes associated with enlargement of the membranous septum (Rosenquist et al. 1975).

Tricuspid Atresia

In this uncommon malformation, there is complete absence of the tricuspid valve, its site being marked by a dimple in the floor of the right atrium that is the membranous septum (Rigby et al. 1990). There is an associated atrial septal defect with all the systemic venous return passing to the left ventricle; the right ventricle is rudimentary and, of necessity, connected to the left ventricle via a VSD that is frequently restrictive (Fig. 21.26). The ventriculoarterial connections are usually concordant, the restrictive VSD thus causing subpulmonary stenosis.

Uhl's Anomaly

This anomaly is a very rare condition of congenital absence of the myocardium of the parietal wall of the right ventricle (Uhl 1952). It presents as cyanosis and congestive heart failure in the neonate (Corazza et al. 1981). The right atrium is hypertrophied and dilated with endocardial thickening. The right ventricle is very dilated and very thin walled. Histologically, it shows absence of myocardium with replacement by fatty tissue. Distinction from arrhythmogenic right ventricular cardiomyopathy depends, to



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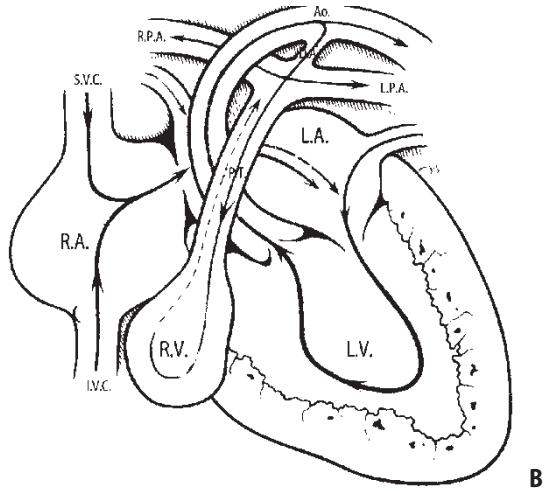


B

Figure 21.26. Atrioventricular valve atresia. (A) The right ventricle is hypoplastic, fibrosed with smooth inner surface, and a wide pulmonary cone and absent pulmonary valves. The tricuspid valve (arrow) is atretic, situated below and behind the infundibulum. The right atrium and appendage are dilated. (B) Schematic diagram illustrating the cardiac blood flow. RAA, right auricular appendage; RA, right atrium; RV, hypoplastic RV; LV, left ventricle; LA, left atrium; Ao, aorta; PT, pulmonary trunk; RPA and LPA, right/left pulmonary arteries.

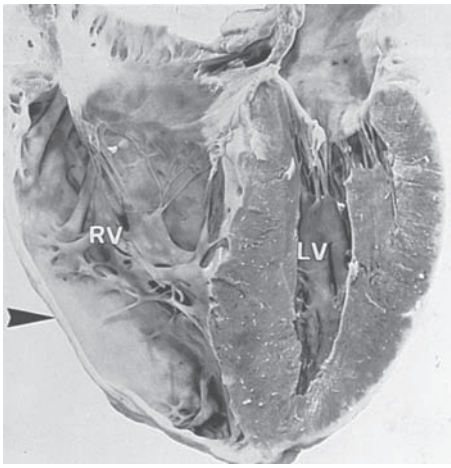


Figure 21.27. Uhl's anomaly. The right ventricular wall is thin, fibrous (parchment) and dilated arrowhead. RV, right ventricle; LV, left ventricle.

some extent, on the age of presentation (Fig. 21.27).

Atrial Isomerism

Atrial isomerism represents complex anomalies of laterality. The normal arrangement of left and right atria is abolished and instead the heart has either two morphologically right atria or two morphologically left atria. It should be stressed that there is still a right-sided atrium and a left-sided atrium, but that both atria have the characteristics of either a morphologically right or of a morphologically left atrium. This recognition of morphologically right or left atrium depends on the appearance of the atrial appendage. As noted in the section on normal anatomy, the right atrium has an appendage that is broad and triangular with a broad junction with its atrium and pectinate muscles that completely surround the AV valve orifice; the left atrial appendage is long and tubular with a distal hook and a narrow junction with its atrium and pectinate muscles confined to the appendage proper.

Atrial isomerism can thus occur in right or left forms. Atrial isomerism may exist without other disturbance of laterality, but frequently occurs with isomerism of the bronchial arrangement. Normally, the right main bronchus is short and eparterial, which means that it gives off its upper lobe branch at the same level as the pulmonary artery. The normal left main bronchus is long and is hyparterial, which means that it divides below the level of the pulmonary artery on that side. In right bronchial isomerism both bronchi are short and eparterial, while in left bronchial isomerism, both bronchi are long and hyparterial. There may be isomerism of other organs (see below). Within the chest the heart is often abnormally positioned and may be on the right, the left, or in the middle, and, likewise, the apex may be directed to the left, the right, or centrally Macartney et al. 1980).

Right Atrial Isomerism

By definition, these hearts show bilateral morphologically right atrial appendages (Fig. 21.28). Usually there are bilateral superior caval veins entering the roof of the atrial chamber. The inferior vena cava may be a central structure or less commonly may be bilateral. The interatrial septum is usually rudimentary. The coronary sinus is absent. The venous drainage is anomalous, to an extracardiac site in most cases. Typically there is double inlet ventricle with a common atrioventricular valve, discordant ventriculoarterial connection, or double outlet ventricle with associated pulmonary stenosis or atresia. There may be right bronchial isomerism with bilateral trilobed lungs, asplenia, and a central liver with symmetrical right and left lobes. The stomach

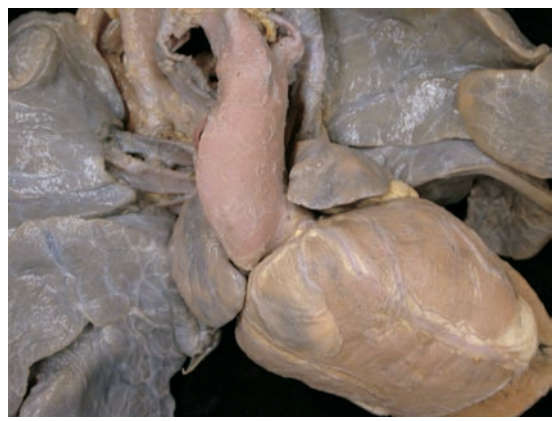


Figure 21.28. Right atrial isomerism. Morphologically right-sided atrial appendages are present on either side of the arterial pedicle. Internally, the pectinate muscles of both appendages extended on the atrial wall around the atrioventricular valve. Bilateral superior caval veins are also visible. (Courtesy of Dr. Andrew Cook, Institute of Child Health, London.)

may be on the left or the right side, and there is intestinal malrotation.

Left Atrial Isomerism

By definition, there are bilateral morphologically left atrial appendages (Fig. 21.29). As in right atrial isomerism, bilateral superior caval veins are usually present. Pulmonary venous drainage is to the atrial mass. Typically, there is interruption of the inferior caval vein with continuation as the azygos vein to the superior caval vein. The hepatic veins drain separately to the atria, sometimes bilaterally. The atrial septum tends to be better formed than in right isomerism. There is usually an atrioventricular septal defect. The ventriculoarterial connection is usually concordant with normal relations of the great arteries. Coarctation or interrupted aortic arch may be present.

The viscera may show left bronchial isomerism with bilobed lungs bilaterally. There may be polysplenia.

Juxtaposition of the Atrial Appendages

In the normal situation, the atrial appendages lie on either side of the great arteries. Sometimes both atrial appendages lie side by side on one side of the great arteries (Fig. 21.30). This situation is termed juxtaposition of the atrial appendages and it is more common for the right atrial appendage to be thus abnormally located. The condition is usually associated with other cardiac malformations, particularly complete transposition (Melhuish and Van Pragh 1968).

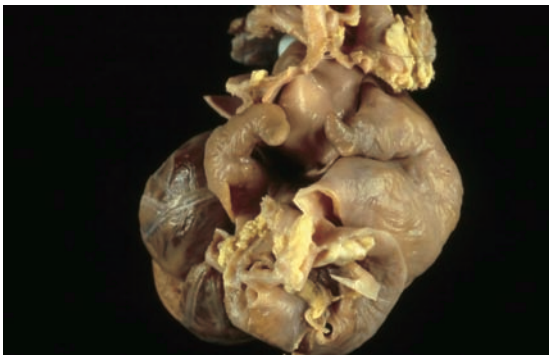


Figure 21.29. Isomerism of the left atrial appendages. The heart is viewed from above to show that there are bilateral atrial appendages of left atrial morphology.

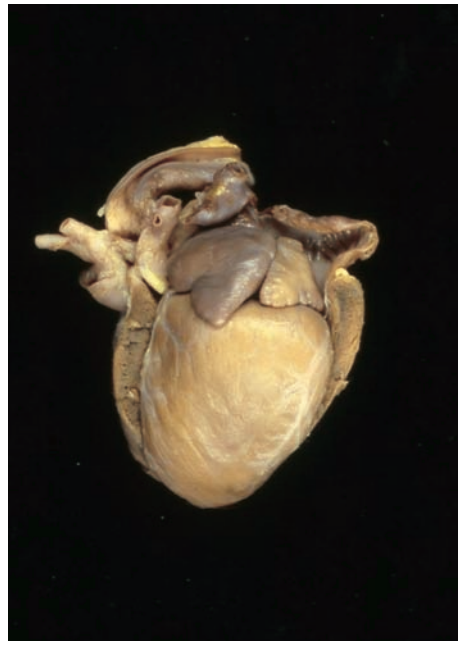


Figure 21.30. Juxtaposition of atrial appendages. The heart is viewed from the left side and shows the right and left atrial appendages lying side by side to the left of the arterial pedicle.

Structural Abnormalities of the Coronary Arteries

In the normal situation, the coronary arteries arise from the right and left facing sinuses of the aortic valve close to the sinotubular junction. The right coronary artery travels downward and to the right to enter the right atrioventricular groove and, in that location, courses around to the posterior aspect of the heart. It supplies branches to the pulmonary infundibulum and to the sinoatrial node, the right ventricular myocardium, and in about 90% of cases supplies the posterior interventricular artery (right dominant circulation). The left coronary artery branches after a course of a few millimeters from its origin from the aorta to give an anterior descending artery and a circumflex branch, the latter traveling in the left atrioventricular groove to supply a variable amount of the left ventricular myocardium. The coronary arteries exit the aorta at right angles to that vessel, and although both main vessels skirt the pulmonary trunk, they are not compressed between it and any other structure. For most of their proxi-

mal course the coronary arteries rest on the epicardial surface of the heart. They may dip down into the myocardium for a variable length and to a variable depth before reemerging onto the epicardial surface. The significance, if any, of this myocardial bridging is debated. It is generally not thought to have any pathological significance, except perhaps in the context of hypertrophic cardiomyopathy (Yetman et al. 1998).

Anomalous Origin of the Coronary Arteries from the Aorta

Some variations to the above pattern occur so frequently as to be part of the normal spectrum. In about 50% of cases, two, sometimes even three, right coronary arteries arise from the right facing sinus (Becker 1981). The extra vessel is usually small, supplying only a small part of the pulmonary infundibulum. There are also numerous variations on one or another artery arising anomalously from the opposite coronary sinus (Neufeld and Schneeweiss 1983). Although there are cases reports of all being associated with an increased incidence of sudden death, two patterns only appear likely to be associated with the potential for vascular occlusion: an oblique course of the anomalous artery through the wall of the aorta such that the expansion of the aorta in systole acts to close the vessel lumen, and an epicardial course of the anomalous artery between the pulmonary trunk and the aorta when the potential exists for compression of the coronary artery between the two great vessels (Ness and McManus 1988).

A single coronary artery arising from the aorta and supplying both right and left coronary circulations is extremely uncommon in an otherwise normal heart (incidence less than 1:7000) (Kimbiris et al. 1978). It is much commoner in association with transposition of the great arteries (Fig. 21.31).

An abnormally high takeoff of the coronary arteries from the aorta, arbitrarily defined as greater than 1 cm above the sinotubular junction, is associated with increased cardiac morbidity, possibly because of an oblique course through the aortic wall.

The situation of disconnection of the coronary arteries from their aortic attachment has already been mentioned in the context of pulmonary atresia with intact ventricular septum.

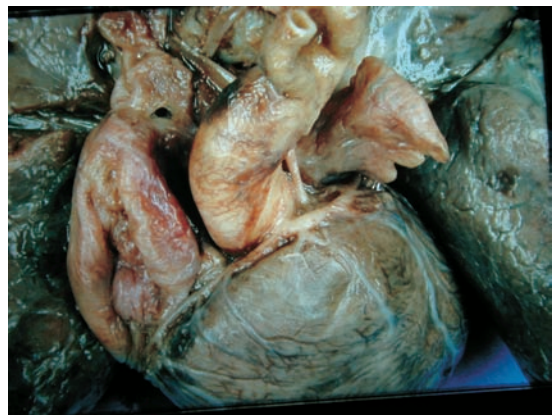


Figure 21.31. Single coronary artery. A single artery leaves the aorta anteriorly and branches almost immediately to give rise to left and right coronary arteries. There is also pulmonary atresia; note the thread-like pulmonary trunk to the left of the aorta.

Anomalous Origin of the Coronary Arteries from the Pulmonary Trunk

Where two coronary arteries arise normally from the aorta, it is possible, occasionally, to see a small supernumerary artery arising from the anterior pulmonary trunk. This vessel is very small, supplies usually only a small part of the anterior right ventricular wall, and is of no grave consequence.

Of much more sinister significance is origin of the left coronary artery from the pulmonary trunk (Arey 1984c) (Fig. 21.32). In utero this anomaly presents no particular problem to the fetus, but in the first weeks of life, as pulmonary vascular resistance falls and with it the pulmonary artery pressure, perfusion of the territory of the anomalously arising artery is seriously compromised. The child presents with crying and sweating on feeding because of myocardial ischemia and infarction and shows signs of cardiac failure or shock. Sudden death may occur. If the child survives this period, possibly because of well-developed collateral circulation, presentation may be at a later age. Collateral development may be so extensive as to result in steal to the pulmonary circulation. The artery usually arises from the posterior leftward pulmonary sinus. Over time the left coronary artery becomes thin-walled and ischemic. Fibrosis develops in the left ventricular myocardium, in association with endocardial fibrosis. There may be mitral regurgitation because of ischemic papillary muscle damage.

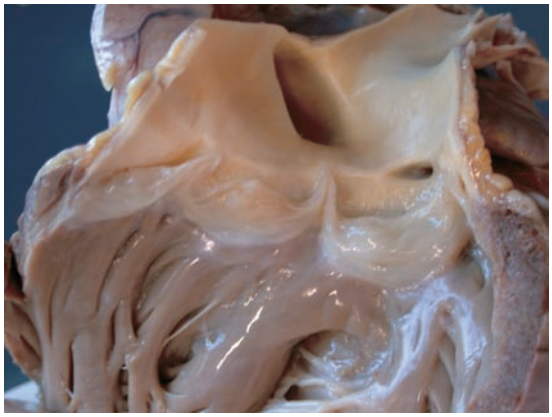


Figure 21.32. Left coronary artery arising from the pulmonary trunk. The right ventricular outflow tract has been opened. The left coronary artery takes origin from the pulmonary trunk. There was extensive myocardial fibrosis in the distribution of the artery.

The right coronary artery may arise from the pulmonary trunk, but the presentation is usually less dramatic.

Other Abnormalities

Persistent Left Superior Vena Cava

This is a relatively frequent occurrence (approximately 0.5% of the general population) (Buirsky et al. 1986), and may occur in an otherwise normal individual. Its incidence is increased in association with congenital heart disease, and it is an almost invariable finding with right atrial isomerism. The vein drains to the coronary sinus and the blood enters the right atrium. There may be absence of the innominate vein in about 40% of cases (Fig. 21.33).

Ectopia Cordis

In this rare malformation the heart lies outside the thoracic cavity. In the most common variety there is a defect of the sternum, pericardium, and overlying skin, and the anteriorly displaced heart lies exposed to the external environment (Fig. 21.34). There are usually associated cardiac and extracardiac defects. A less common variation is when the heart lies in the upper abdomen, usually associated with a defect of the lower sternum, diaphragm, pericardium, and upper abdominal wall with protrusion of both heart and abdominal contents onto the body surface. There are usually associated cardiac defects, most commonly VSD (Cantrell et al. 1958). This combination of abnormalities is sometimes termed pentad (or pentalogy) of Cantrell.

Structural Heart Disease in the Fetus

In a normal population, the risk of a woman having a child with a congenital heart malformation is 0.8% to 1% (Hoffman and Kaplan 2002). If a previous child has had a heart defect, the risk rises to 2% to 3% (Gill et al. 2003), and if the mother herself has a congenital heart defect, the risk approaches 6%.

There is considerable variation among countries in antenatal detection of heart defects; the lowest detection rates are in those countries

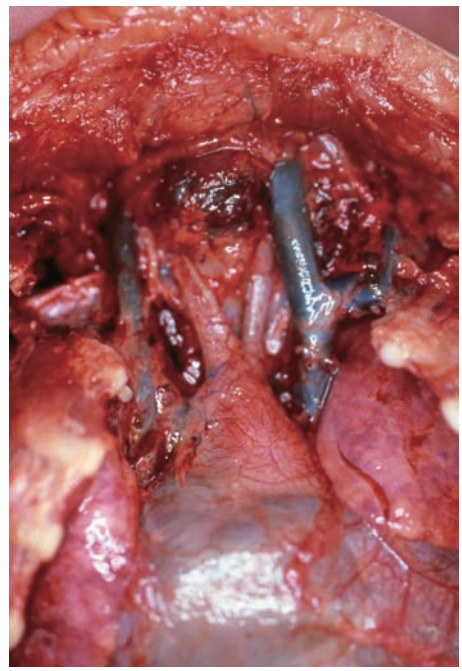


Figure 21.33. Persistent left superior caval vein. The thymus has been removed and the pericardium remains intact. There are bilateral superior caval veins. The left was connected to the coronary sinus on the posterior aspect of the left atrium. In this case there is absence of the innominate vein, but it may be present with a left superior caval vein.

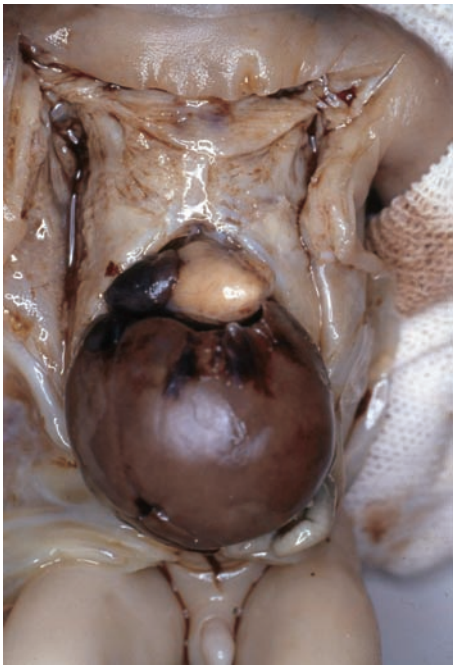


Figure 21.34. Ectopia cordis. The heart is present in an abnormal location beneath the shortened sternum (Courtesy of Dr. Helen Porter, Leicester, England.)

without ultrasound antenatal screening, at around 8% to 11%. In Western Europe, where ultrasound screening is routine, the detection rates vary between 19% and 48%. Detection depends crucially on the skill of the operator and is highest when a cardiologist with fetal ultrasonographic experience performs the examination. High-resolution echocardiography enables assessment of precise structures during the second trimester.

In about half the cases detected antenatally, the pregnancy is terminated; termination is more likely if there are associated malformations or chromosomal abnormality (Rodriguez et al. 1998). Some heart defects may not be detected early on, but may progress during pregnancy. This has been well documented with obstructive right- and left-sided lesions (Hornberger et al. 1995; Maeno et al. 1999). The defects most likely to be detected are those that affect the size of the ventricles or cause in-utero heart failure. Thus, the spectrum of heart lesions seen in cases of termination of pregnancy is different from that seen in liveborn children (Allan et al. 1994). The death rate in utero for cases with congenital heart disease is high, which contributes to the difference between the populations of liveborn and non-liveborn (Eronen 1997). Does detection of structural heart disease in utero affect fetal outcome? Data are beginning to emerge to suggest that in those cases without chromosomal abnormality or other associated defects, detection of heart disease in utero can improve outcome (Yates 2004).

Although nearly every form of heart defect has been described in association with fetal hydrops (see Chapter 14), in fact most fetus with heart defects do not develop hydrops. It is only those with a severe mechanical defect or those with arrhythmia who do so (McFadden and Taylor 1989). The practical implication of this is that there must be very compelling evidence to ascribe hydrops to a cardiac defect that in any particular case is more likely to be coincidental. Hydrops with intrauterine death has been described in association with absence of the ductus venosus, and that structure should be examined carefully in all cases of fetal hydrops (Siven et al. 1995).

Inflammation

Infectious Endocarditis

Infectious endocarditis is infection confined to the endocardium and the immediately subjacent structures. It usually affects the valves of the heart or the immediately surrounding endocardium. It may affect the edges of ventricular septal defects, patent ductus arteriosus, coarctation, indwelling catheters, or prosthetic material within the heart. There is usually underlying valvular pathology, but with potential pathogens, endocarditis can occur in a healthy valve. In the setting of congenital heart disease, VSD, obstruction of the left ventricular outflow tract, tetralogy of Fallot, and tricuspid atresia are the most common underlying lesions (Zuberbuhler et al. 1994). The most common microorganisms are *Streptococcus viridans* and *Staphylococcus aureus* (Fowler et al. 2005). Less frequent organisms are coagulase-negative staphylococci and *Candida* sp. Pneumococcal endocarditis is an aggressive disease with a high mortality (Choi and Mailman 2004).

The vegetations tend to occur on the right side of the heart, predominantly the tricuspid valve, and in the setting of a VSD, on the right side of the defect. Associated with coarctation they may be on the aortic valve or in the aorta just distal to the coarctation. With a patent arterial duct the vegetations are usually in the left pulmonary artery adjacent to the duct (Arey 1984b). Histologically, the vegetations consist of fibrin, necrotic material, platelets, neutrophil polymorphs, and bacterial colonies. Older vegetations become fibrotic and calcified. There is variable destruction of the underlying valve architecture. The damage done to the heart is a result of valvular regurgitation and that to vessels is the formation of aneurysms or occlusion by embolus.

Nonbacterial thrombotic endocarditis can occur in neonates, usually on the tricuspid valve in association with persistent pulmonary hypertension of the newborn. The vegetations may be quite large and small thrombi are often to be found in the smaller pulmonary arteries (Favara et al. 1974; Morrow et al. 1982) (Fig. 21.35).

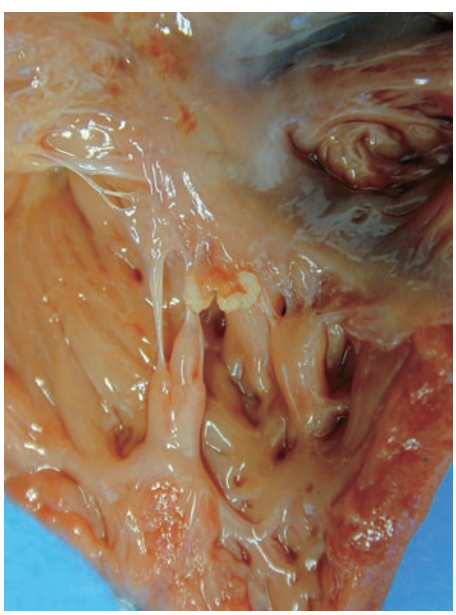


Figure 21.35. Nonbacterial thrombotic endocarditis. The free edge of the anterosuperior leaflet of the tricuspid valve shows nodular, pale excrescences. The infant had persistent pulmonary hypertension of the newborn.

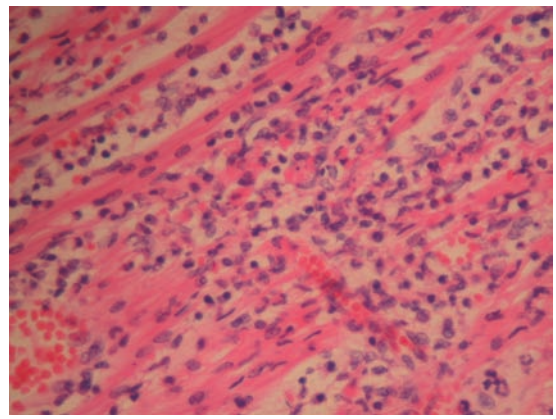


Figure 21.36. Viral myocarditis. The myofibers of the interventricular septum are separated by edema, and there is an infiltrate of lymphocytes, macrophages, and neutrophil polymorphs. Some myofibers are degenerate, and there is interstitial hemorrhage. Coxsackie virus was cultured from blood and myocardium.

Myocarditis

Myocarditis is defined as an inflammatory infiltrate of the myocardium with necrosis or degeneration of adjacent myocytes, not typical of the ischemic injury associated with coronary artery disease (Fig. 21.36). Myocarditis can be caused by drugs or an immunological reaction, but most cases are infectious in origin with viral myocarditis being by far the commonest category (Feldman and McNamara 2000). During many viral illnesses, a transient lymphocytic infiltrate may be seen within the myocardium sometimes associated with mild functional disturbance. The commonest viruses causing myocarditis are Coxsackie B viruses (Kim et al. 2001). Infection with these viruses is particularly common in infants. Approximately 20% of cases of dilated cardiomyopathy show evidence of Coxsackievirus RNA (Baboonian et al. 1997) by polymerase chain reaction (PCR). Other viruses causing myocarditis are influenza virus, human immunodeficiency virus (HIV), cytomegalovirus, and adenovirus type 2. Clinical myocarditis depends on a complex interplay between the infecting virus and the T-cell response of the host.

Cardiovascular problems associated with HIV infection, including left ventricular dysfunction and increased left ventricular mass, are common and clinically important indicators of survival for children with HIV (Keesler et al. 2001). However, it remains unclear whether HIV directly infects

the myocardium or myocarditis is due to other opportunistic viruses (Bowles et al. 1999).

In-utero infection with parvovirus B19, while usually causing anemia leading to hydrops, may cause myocarditis by direct infection of the myocardium with resulting hydrops and intrauterine death (O'Malley et al. 2003). Parvovirus B19 infection is also being recognized increasingly as a cause of myocarditis and cardiac dysfunction in children (Munro et al. 2003).

Bacterial myocarditis in the absence of endocarditis, while rare, does occur, usually in the setting of overwhelming bacteremia (Wasi and Shuter 2003). The leading bacterial pathogen is *S. aureus*. There are multiple small abscesses in the myocardium, usually of the left ventricle. Disturbance of cardiac contraction or of rhythm may occur, or there may be rupture into the pericardium with development of suppurative pericarditis.

Toxoplasma may cause myocarditis following maternal infection and transplacental passage. Myocardial necrosis, scarring, and calcification have been described (Rosenberg 1987). There are usually associated brain abnormalities, and pseudocysts may be seen in the placenta or even in the myocardium.

Pericarditis

Viral pericarditis usually occurs in the setting of viral myocarditis and may overshadow the myocardial involvement. Pericardial effusions may develop following the use of central venous catheters in premature infants. The tip of the catheter may press against the atrial wall; the atrial wall is extremely thin and fluid exudes through it into the pericardial cavity, on occasions causing fatal tamponade (Beardsall et al. 2003).

Rheumatic fever is uncommon in children under 5 years of age, and is exceptionally rare before the age of 1 year (Tani et al. 2003), and so is not discussed further here.

Inflammatory conditions of the coronary arteries are discussed below.

Myocardial Ischemia and Infarction

Myocardial necrosis secondary to ischemia is by far the most common form of injury to the myocardium in the perinatal period. It is caused by low rates of perfusion rather than by an acute occlusive episode, and the asphyxiated infant is particularly vulnerable. In the fetus, the coronary arteries can now be visualized and studied with high-resolution ultrasound and sensitive color Doppler technology (Chaoui 2004).

The heart is the organ with the highest oxygen consumption for its weight in the body. In response to fetal hypoxia the coronary perfusion can be increased by a factor of three to five, the so-called heart-sparing effect. There are indications that this is a late stage of compensation and that myocardial damage may already have occurred at this stage (Makikallio et al. 2002). Asphyxiated infants are at high risk of neonatal cardiovascular insufficiency. Premature infants with chronic lung disease, infants with persistent pulmonary hypertension of the newborn, and infants with right and left heart obstructive lesions are also susceptible to myocardial ischemia. The papillary muscles and the subendocardial zone are the most likely to be affected (Young et al. 1994), but the injury may be patchy. The severity ranges from microscopic foci of ischemic necrosis to frank, macroscopically evident, myocardial infarction (DeSa 1979) (Fig. 21.37A). Dystrophic calcification is particularly common (Topaz 1991) (Fig. 21.37B). Foci of calcification in the papillary muscles of both right and left ventricles also occur in fetus with trisomies 21 and 13, but may also occur in otherwise normal fetus (Tennstedt et al. 2000); the pathogenesis is not determined.

Anomalous origin of the left coronary artery from the pulmonary trunk results in infarction of the supplied myocardium. Left ventricular ischemia may also occur following shock or bradycardia associated with necrotizing enterocolitis or severe cerebral hemorrhage in the premature infant. Infarction of the interventricular septum usually involves the upper part, and the atrioventricular conduction tissue may be affected. As in adults, it takes up to 12 hours following the ischemic insult for changes to appear in the myocardium. The affected myofibers become swollen and show eosinophilic granularity of their cytoplasm with loss of the usual striations. The nuclei become pyknotic and disappear. There may be associated extravasation of erythrocytes. Contraction band necrosis is uncommon. There may be calcification of individual necrotic myocytes. Fibrin or platelet thrombi may be found in capillaries or other small

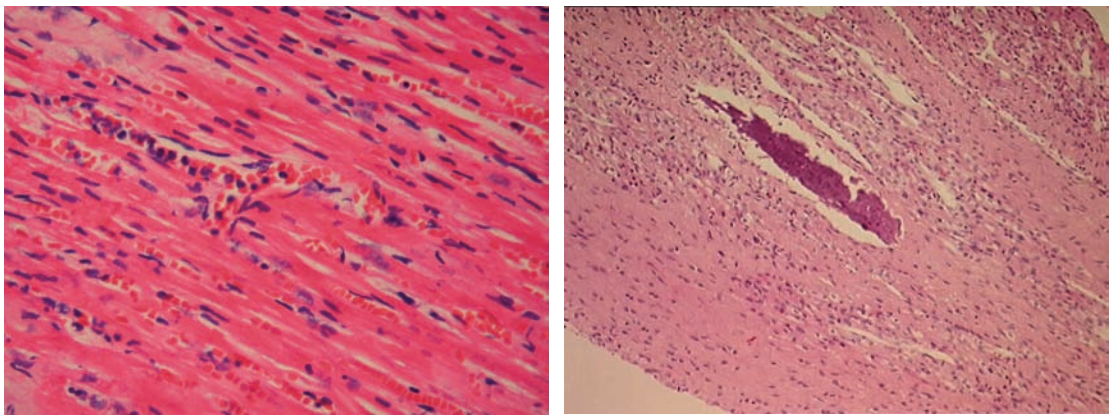


Figure 21.37. (A) Myocardial necrosis left ventricle. The myofibers are hypereosinophilic with floccular cytoplasm and the nuclei are smudgy. A few fragments of nuclear debris are visible. There is capillary congestion. The infant died at age 68 hours following cesarean section for fetal distress. The brain showed extensive hypoxic neuronal necrosis. (B) Calcification mitral valve papillary muscle from a case of hypoplastic left heart. There is extensive surrounding myofiber loss with replacement fibrosis.

vessels in the ischemic area. Immunohistochemistry for the C9 complement component may demonstrate myofiber damage at an earlier stage of its development than coagulative necrosis and may be used in macerated stillbirths (Lazda et al. 2000). Immunohistochemical staining for cardiac troponins T and I appears to hold similar promise (Fishbein et al. 2003).

Cardiomyopathy

The term *cardiomyopathy* denotes disease of the heart muscle and excludes disease secondary to congenital malformations. The World Health Organization has classified cardiomyopathy into several types on the basis of the dominant pathophysiology or by etiological/pathogenetic factors (Table 21.2) (Richardson et al. 1996). The pathology of the main types tends to be distinctive, but there are areas of overlap; late in their course, some forms of hypertrophic cardiomyopathy may develop a dilated phenotype, and most forms of dilated and restrictive cardiomyopathy show increased heart weight and histological evidence of myofiber hypertrophy. The etiology of some cardiomyopathies is known, for example, cardiomyopathy secondary to metabolic disorder or in association with muscular dystrophy or other skeletal muscle disease. Many forms of cardiomyopathy have a genetic or familial basis, but some forms of heart muscle disease are undoubtedly acquired as a result of exposure of susceptible individuals to infectious agents or toxins. There may be no recognized factor to which the heart muscle disease is attributable—so-called primary or idiopathic cardiomyopathy. This classification is in need of revision as the genetics and pathology of the underlying diseases are increasingly understood, but it still provides a useful framework within which to discuss heart muscle disease. Metabolic disorders affecting the heart are listed in Table 21.3.

Hypertrophic Cardiomyopathy

As originally described, this is a disease of the heart muscle where the primary pathology is hypertrophy of the ventricular myocardium in the absence of a predisposing factor such as systemic hypertension or valvular heart disease, often with asymmetric involvement of the interventricular septum and sometimes associated with obstruction of the left ventricular outflow (Wigle et al. 1995). Its histological hallmark is myofiber disarray, and there are frequently dysplastic changes in the intramyocardial arteries (Fig. 21.38). The disease was originally described in adolescents and was associated with a high frequency of sudden death. It is now known that this can occur in neonates and even in utero.

Most cases of idiopathic hypertrophic cardiomyopathy are now known to be the result of mutations in the genes encoding structural proteins of the contraction apparatus of the cardiac myofiber (Redwood et al. 1999). To date, mutations in 11 genes coding for cardiac sarcomeric proteins have been found to cause hypertrophic cardiomyopathy (Ahmad et al. 2005). Among these genes are the genes encoding the heavy chain of β-myosin, actin, tropomyosin, and cardiac troponins T, C, and I. It is also recognized that particular mutations may correlate with a particular phenotype and correspond with, for example, the degree of myofiber hypertrophy or the magnitude of the risk of sudden death (McKenna et al. 1998).

Although myofiber disarray is the histological hallmark of hypertrophic cardiomyopathy, it can occur in other settings. Myofiber disarray occurs in the normal heart and, if sought, can be found at the junction of the free wall of the ventricles with the interventricular septum. The region affected, however, is small, and disarray should not be seen in the normal heart in either the interventricular septum or lateral ventricular walls. Myofiber disarray may be seen in many forms of myocardial hypertrophy, and it is present in the hypertrophy accompanying many forms of congenital heart disease, most notably hypoplastic left heart where it is described in up to 80% of

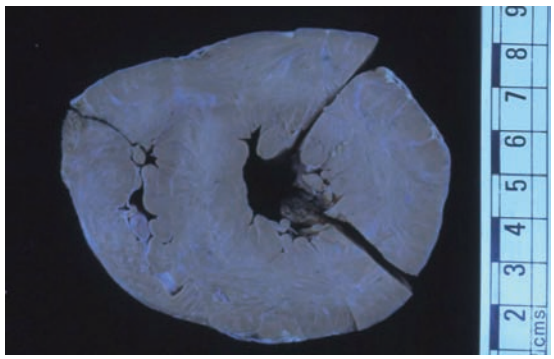
Table 21.2. Classification of cardiomyopathies

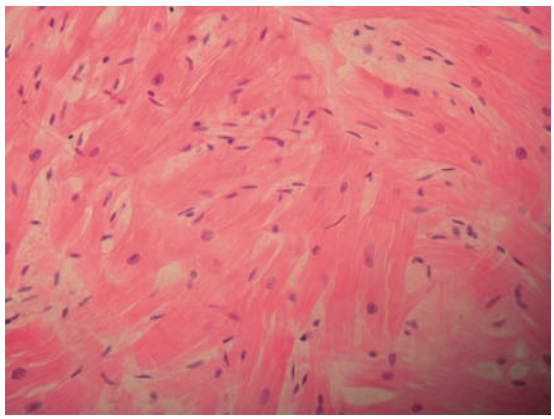
Dilated cardiomyopathy	
Hypertrophic cardiomyopathy	
Restrictive cardiomyopathy	
Arrhythmogenic right ventricular cardiomyopathy	
Unclassified	
Noncompacted myocardium	
Fibroelastosis	
Mitochondrial	
Specific cardiomyopathies	
Ischemic cardiomyopathy	
Valvular cardiomyopathy	
Hypertensive cardiomyopathy	
Inflammatory cardiomyopathy	
Metabolic cardiomyopathy	
Includes:	*Endocrine,* e.g., thyrotoxicosis, hypothyroidism, adrenal cortical insufficiency, pheochromocytoma, acromegaly, and diabetes mellitus
	Familial storage disease and infiltrations, e.g., hemochromatosis, glycogen storage disease, Hurler's disease, Refsum's syndrome, Niemann-Pick disease, Hand-Schüller-Christian disease, Fabry-Anderson disease, and Morquio-Ullrich disease
	Deficiency, e.g., disturbances of potassium metabolism, magnesium deficiency, and nutritional disorders such as kwashiorkor, anemia, beriberi, and selenium deficiency
	Amyloid, e.g. primary, secondary, familial and hereditary cardiac amyloidosis, familial Mediterranean fever, and senile amyloidosis
General system disease	
Includes:	*Connective tissue disorders,* e.g., systemic lupus erythematosus, polyarteritis nodosa, rheumatoid arthritis, scleroderma, and dermatomyositis
	Infiltrations, e.g. sarcoidosis, leukemia
Muscular dystrophies	
Includes:	Duchenne, Becker type, and myotonic dystrophy.
Neuromuscular disorders	
Includes:	Friedreich's ataxia, Noonan syndrome, and lentiginosis
Sensitivity and toxic reactions	
Includes:	Reactions to alcohol, catecholamines, anthracyclines, irradiation
Peripartal cardiomyopathy	

Source: Adapted from World Heath Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies.

Table 21.3. Metabolic, storage, and other diseases secondarily affecting the heart in the fetus and infant (Pernot et al. 1978; Miranda et al. 1979; Kelley et al. 1991; Hayflick et al. 1992; VanNoort et al. 1993; Cochat et al. 1999; Gilbert-Barness 2002; Gehrmann et al. 2003)

Metabolic disorders
Medium chain acyl co-A dehydrogenase deficiency
Long-chain hydroxy acyl co-A dehydrogenase deficiency
Deficiencies of carnitine, carnitine acylcarnitine translocase and carnitine palmitoyl transferase 1
Congenital disorders of glycosylation
Primary hyperoxaluria type 1
Storage disorders
Glycogenosis II (Pompe)
III
IV (Andersen's)
V
Mucopolysaccharidosis 1 (Hunter)
2 (Hurler)
3 (Sanfillipo)
Mitochondrial disorders
Other
Duchenne muscular dystrophy
Friedreich's ataxia
Myotubular myopathy
Nemaline myopathy
Noonan syndrome
Barth syndrome
Hemochromatosis





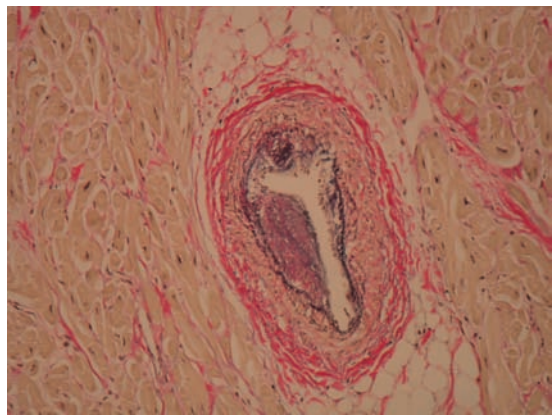


FIGURE 21.38. Hypertrophic cardiomyopathy. (A) Short-axis view of the ventricular myocardium showing concentric left ventricular hypertrophy. The myocardium has a whorled appearance and there are pale streaks of fibrous tissue. There is also right ventricular hypertrophy. (B) Myofiber disarray. The myofibers are enlarged, splayed, and run in different directions. There is increased intervening fibrous tissue. (C) Dysplastic intramyocardial artery. The media is irregularly thinned and the intima shows eccentric fibroelastic thickening.

cases. Myofiber disarray may also be seen in restrictive cardiomyopathy.

The muscle fiber hypertrophy with disarray in hypertrophic cardiomyopathy affects not just the ventricles but can also be found in the atrial myocardium. The identification of hypertrophic cardiomyopathy has important implications for other siblings and family members, and, where possible, genetic material should be obtained at autopsy to permit gene screening.

The term *hypertrophic cardiomyopathy* is now largely restricted to those genetic cardiomyopathies with mutations of the genes encoding sarcomeric structural proteins and that histologically exhibit myofiber disarray. In clinical practice, however, some other cardiomyopathies present with a hypertrophic phenotype. One of these is Pompe disease. Pompe disease may show subaortic stenosis and endocardial fibroelastosis and is usually fatal in the first year of life. Type III glycogenosis may present in infancy with myocardial hypertrophy (Servidei et al. 1994). In contradistinction to the hypertrophic cardiomyopathy caused by mutations in the sarcomeric protein genes, that associated with glycogen storage tends to be associated with abnormal electrophysiology. Histologically, there is vacuolar change in the myocytes with accumulation of glycogen. Myofiber disarray and myocardial fibrosis are not found. A similar histological appearance may be seen with mutations to the *PRKAG2* gene encoding the γ subunit of adenosine monophosphate (AMP)-activated protein kinase (Arad et al. 2002). This produces a hypertrophic phenotype with ventricular preexcitation and progressive conduction system dysfunction; there is no myofiber disarray or fibrosis, but there is myofiber vacuolation and glycogen accumulation. By contrast with the other glycogen storage cardiomyopathies, there are no extracardiac manifestations. The disorder is confined to adults. Mutations in the lysosomal associated membrane protein 2 *(LAMP2)* gene can also cause a hypertrophic cardiomyopathy with glycogen accumulation. Usually there are other systemic manifestations but these may be subclinical and hypertrophic cardiomyopathy

may be the presenting feature. Although the disorder is described in children as young as 8 years, it has not been reported in the neonatal period (Arad et al. 2005).

Friedreich's ataxia and Noonan syndrome may also present with a hypertrophic cardiomyopathy phenotype (Dutka et al. 1999). Noonan syndrome is also associated with polyvalvular dysplasia and pulmonary stenosis (Sreeram et al. 1994). About 45% of cases of Noonan syndrome are due to mutations in the *PTPN11* gene (Tartaglia and Gelb 2005).

Infants of diabetic mothers may develop myocardial hypertrophy identical to hypertrophic cardiomyopathy in the neonatal period (McMahon et al. 1990); this cardiomyopathy is usually transient and resolves spontaneously. Hypertrophic cardiomyopathy is also described in infants who have received steroids for pulmonary immaturity or chronic lung disease (Israel et al. 1993); again, this cardiomyopathy tends to be transitory but shows identical echocardiographic appearances and hemodynamics to idiopathic hypertrophic cardiomyopathy.

Dilated Cardiomyopathy

This describes the phenotype of biventricular dilatation with atrial dilation and histological evidence of myocardial hypertrophy and fibrosis (Gilbert-Barness and Barness 1999). There is frequently associated endocardial fibroelastosis in the ventricles and sometimes atria, which, in some cases, can be quite marked. The heart has a globular shape (Fig. 21.39), and internally muscular trabeculae, including the papillary muscles of the atrioventricular valves, appear stretched and thinned. As with the other cardiomyopathies, this dilated form represents a phenotype with a wide variety of causes. Viral myocarditis may progress to dilated cardiomyopathy, and some forms of dilated cardiomyopathy yield virus on culture or PCR. It has been shown that enterovirus proteases cleave dystrophin in vitro (Badorff et al. 1999). Many of the skeletal muscle disorders can affect the heart, producing dilated cardiomyopathy, for example, muscular dystrophy (Zatuchni et al. 1961), and the metabolic and mitochondrial cardiomyopathies most frequently display a dilated phenotype (Schwartz et al. 1996). Some dilated

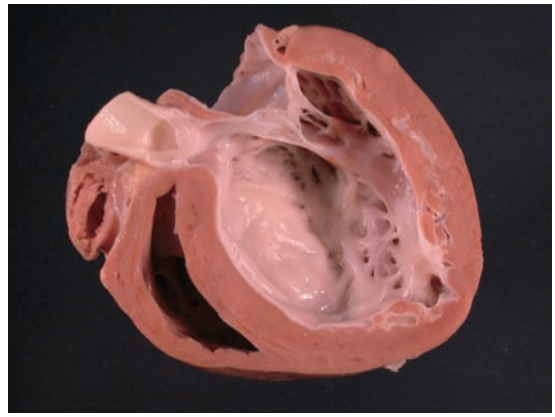


Figure 21.39. Dilated cardiomyopathy. A simulated long-axis view of the heart to show the globular dilatation of the left ventricle with marked left ventricular endocardial thickening.

cardiomyopathies have been shown to be due to mutations in sarcomeric structural protein genes (Graham and Owens 1999; Kamisago et al. 2000). Exclusively breast-fed infants of vitamin D deficient mothers may themselves suffer vitamin D deficiency with development of rickets and may present with dilated cardiomyopathy, which rarely may be fatal. The condition resolves on administration of vitamin D (Price et al. 2003).

Histologically, dilated cardiomyopathy shows fibers with enlarged, hyperchromatic, and irregular nuclei. Many fibers appear stretched, thin, and wavy. There is usually a considerable degree of interstitial fibrosis. The intramyocardial vessels are usually normal, although in areas of dense scarring they may show intimal fibroelastic thickening. There is usually associated thick endocardial fibroelastosis in both ventricles and atria.

Restrictive Cardiomyopathy

This form of cardiomyopathy is physiologically different from the other two main forms of cardiomyopathy (Kushwaha et al. 1997). It accounts for 2% to 5% of cardiomyopathies in childhood (Russo and Webber 2005). The defining characteristics are a stiff ventricular myocardium with diastolic dysfunction. This leads to high diastolic pressures and dilatation of the atria (Fig. 21.40). Clinically, restrictive cardiomyopathy mimics constrictive pericarditis (Hughes and McKenna

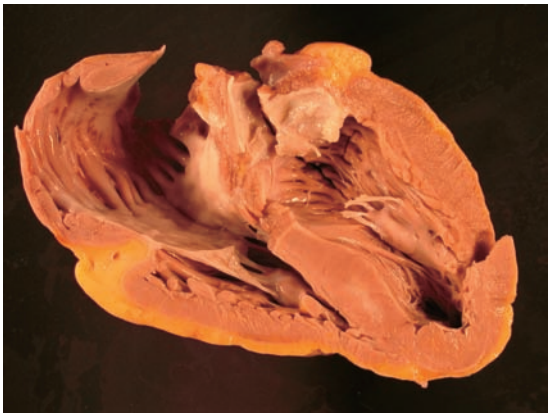


Figure 21.40. Restrictive cardiomyopathy. There is hypertrophy of the myocardium. The most striking feature is the marked dilatation of the right atrium. The interventricular septum shows fibrosis. An explanted heart, the arterial valves have been harvested.

2005). The high left-sided diastolic pressure leads to congestive vasculopathy in the lungs and the development of pulmonary hypertension. Histologically, the myofibers are hypertrophied, and there may be myofiber disarray and prominent interstitial fibrosis, sometimes with a pericellular distribution. Cardiac involvement by amyloid may result in a restrictive cardiomyopathy, but this is very rare in children. Similarly, myofibrillary myopathy, a form of myopathy that shows characteristic myocyte inclusions in association with skeletal myopathy and restrictive cardiomyopathy, does not present in infancy (Selcen et al. 2004). Glycogen storage disorders and the mucopolysaccharidoses may present as restrictive cardiomyopathy, as may primary endocardial fibroelastosis. Secondary endocardial fibroelastosis generally does not.

Arrhythmogenic Right Ventricular Cardiomyopathy

This disorder, characterized by fatty and fibrous replacement of the right (and sometimes the left) ventricular myocardium and life-threatening ventricular tachyarrhythmias (D'Amati et al. 2001), usually presents in the teenage years or early twenties. It does not occur in the neonatal period, although it does have some phenotypic overlap with Uhl's anomaly.

Noncompaction of the Ventricular Myocardium

This is a form of dilated cardiomyopathy of unknown cause that is not widely recognized but that demonstrates a striking morphology (Fig. 21.41). It affects predominantly the left ventricular myocardium, imparting a spongy appearance (Burke et al. 2005). The appearance is reminiscent of the developing embryonic heart. The ventricular cavity extends almost to the epicardial surface among a myriad of thin muscular trabeculations; the normal compact layer separating the muscular trabeculations of the ventricular lining from the epicardium is reduced in thickness. The papillary muscles of the mitral valve are poorly developed. There is quite often prominent endocardial fibroelastosis. Histologically, there are anastomosing endocardial-lined recesses that extend deeply into the myocardium. There may be foci of subendocardial fibrosis in infants older than a few weeks. In children, half the cases show associated cardiac abnormalities such as VSD, polyvalvar dysplasia, and pulmonary stenosis. The case may present in the neonatal period with cardiac failure or with sudden death, although other cases may not present until later in life. Adults tend not to have associated cardiac abnormalities. Syndromes associated with ventricular noncompaction are Barth syndrome (Bleyl et al. 1997), a

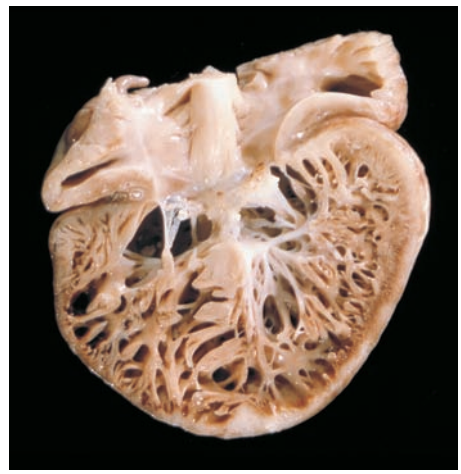


Figure 21.41. Noncompaction. The ventricular wall is thickened and present very deep intertrabecular spaces communicating with the cavity consistent with noncompaction (spongy) myocardium.

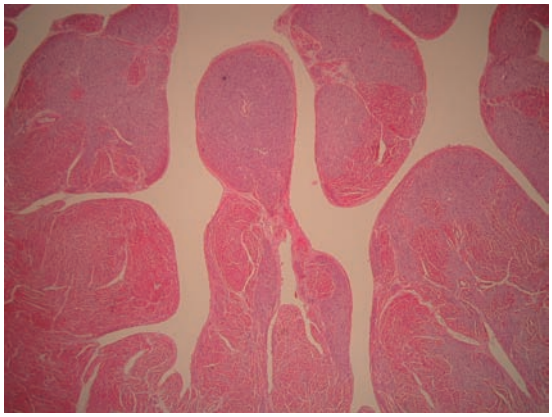


Figure 21.42. Histiocytoid cardiomyopathy. A low-power view of the left ventricle, showing the multiple nodules of histiocytoid cells beneath the endocardium. The normal myocardium is more eosinophilic. The infant had multiple cardiac arrhythmias before death.

probable genetic syndrome, Di George syndrome (Pignatelli et al. 2003), and Melnick-Needles syndrome (Wong and Bofinger 1997), a connective tissue disorder.

Histiocytoid Cardiomyopathy

This rare, X-linked condition occurs usually in female infants and is characterized by severe, sometimes fatal, arrhythmia. Its histological hallmark is collections of myocytes with vacuolated cytoplasm that resemble histiocytes (Malhotra et al. 1994). Ultrastructurally these cells contain large numbers of mitochondria (Fig. 21.42). Macroscopically, the heart may appear enlarged but is otherwise unremarkable, or there may be multiple nodules in the myocardium. These nodules, which range in size from a few millimeters to over 1 cm, may be subendocardial or scattered throughout the myocardium and may even occur in the valves. The condition may present in utero with tachyarrhythmia and heart failure, or presentation may be delayed until after birth to the age of 4 years when the presenting features may be arrhythmia, seizures, heart failure, cyanosis, or sudden death. Structural heart disease, such as VSD or hypoplastic left heart, may be present (Shehata et al. 1998). The condition is regarded as hamartomatous and of Purkinje cell origin; there is a report of a case associated with mitochondrial DNA mutation (Vallance et al. 2004). Histiocytoid cardiomyopathy has been reported in numerous cases of MLS (microphthalmia and linear skin defects) syndrome (also known as microphthalmia, dermal aplasia and sclerocornea [MIDAS] syndrome), a disorder in which there is deletion of the p22 region of the X chromosome (Bird et al. 1994), suggesting that the gene associated with histiocytoid cardiomyopathy lies in that region of the X chromosome.

Mitochondrial Cardiomyopathies

These are disorders of the heart presenting as cardiomyopathy in which the basic defect is mutation of genes of mitochondrial oxidative phosphorylation. These cardiomyopathies are often associated with neurological disorders such as MELAS (myopathy, encephalopathy, lactic acidosis, stroke), MERRF (myoclonic epilepsy, ragged red fibers), Leigh syndrome, or Kearns-Sayre syndrome. The cardiac mitochondria are more numerous than usual and show abnormalities of size or structure with abnormal internal structure (Marin-Garcia and Goldenthal 1997; Marin-Garcia et al. 1997) (Fig. 21.43).

Clinically, infants with mitochondrial cardiomyopathy show failure to thrive, lactic acidosis, and cardiomegaly. Hypertrophic cardiomyopathy is commoner in infants than dilated cardiomyopathy. Histologically, the myocytes may appear

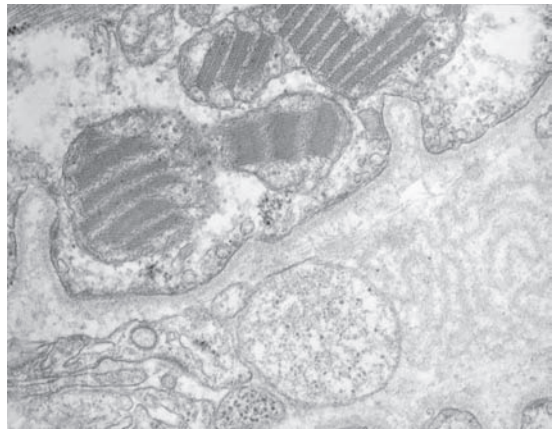


Figure 21.43. Mitochondrial cardiomyopathy: enlarged cardiac mitochondria with few dense bodies and numerous para-crystallin structures in case of mitochondrial disorder involving skeletal and heart muscle.

swollen, with perinuclear clearing and replacement of cross-striations by fine eosinophilic granules representing increased numbers of mitochondria. Staining of frozen sections of myocardium may show reduction in oxidative enzymes, and electron microscopy may show abnormal mitochondria (Taylor 2004).

Metabolic Cardiomyopathy

The types of metabolic disorders that may involve the heart, with clinical features of cardiomyopathy, are listed in Table 21.3.

Tumors

Primary cardiac tumors in infants are rare, with a reported incidence of 1 in 10,000 infant autopsies (Nadas and Ellison 1968), and are usually rhabdomyomas, fibromas, or teratomas. Malignant tumors are extremely rare, and only a few cases have been reported (Burke and Virmani 1995). Symptoms depend on the location and the size of the tumor: murmur, arrhythmia, cyanosis, respiratory distress, and cardiac failure are the presenting features in the neonatal period; arrhythmia, cardiac failure, and hydrops are the presenting features in utero (Isaacs 2004). Thus, despite their benign histology, these tumors may have significant morbidity and even mortality (Sallee et al. 1999).

Rhabdomyoma

Rhabdomyoma is the commonest fetal and neonatal primary cardiac tumor (Beghetti et al. 1997) (Fig. 21.44). It may be single or multiple. If multiple, it is much more likely to be associated with tuberous sclerosis, but not invariably so

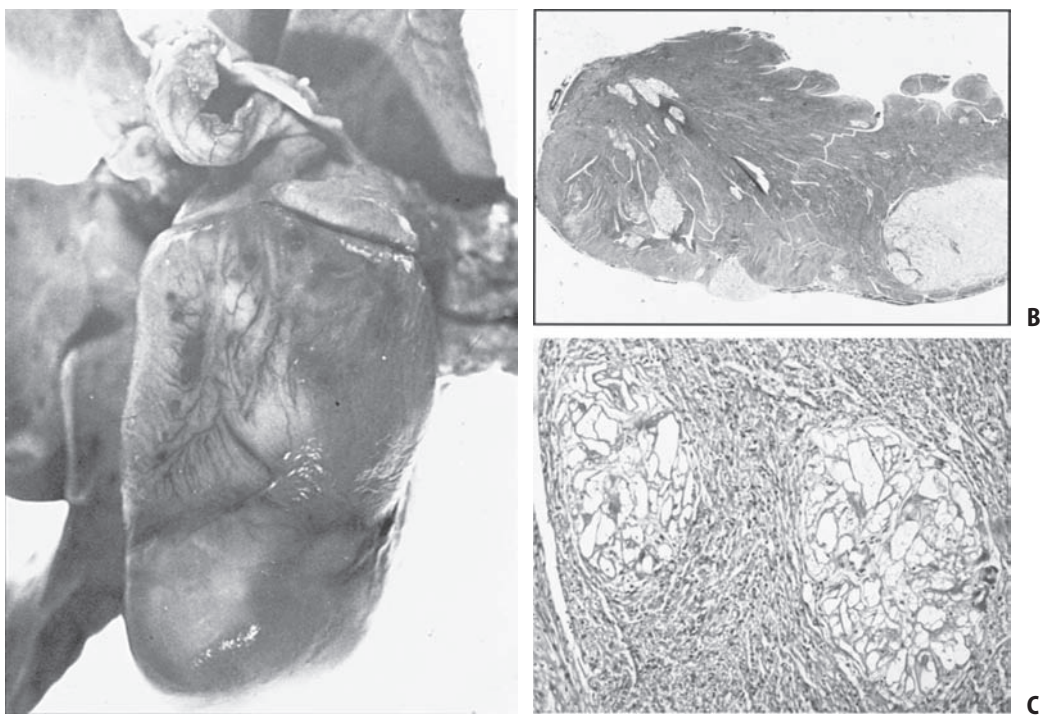


Figure 21.44. (A) Rhabdomyomas of the ventricular myocardium in a neonate. (B) Rhabdomyomas of different size within the myocardium. (C) Glycogen-filled tumor cells vary in size but have a characteristic spider cell appearance.

(Tworetzky et al. 2003). Spontaneous regression is well documented, and resection is not warranted unless there is persistent arrhythmia or hemodynamic compromise. The tumors are well circumscribed and lie within the myocardium, sometimes bulging into the ventricular cavity and causing obstruction of the ventricular outlet. Histologically, the tumor is composed of large cells with clear cytoplasm. The nucleus is connected by strands of cytoplasm to the cell membrane, giving rise to so-called spider cells. There is a variable amount of fibrous tissue dividing the tumor into lobules and there may be small foci of calcification. Histiocytoid cardiomyopathy may cause confusion because of its multiple nodules. More recently an entity called localized nodular hypertrophy has been described in the right ventricular myocardium of a 20- to 21-week nonmacerated stillbirth (Tehrani et al. 2004). The significance of this report is, as yet, unclear.

Fibroma

Fibroma is the second most common tumor (Burke et al. 1994) (Fig. 21.45). It is almost always single. It is generally located in the interventricular septum, but may be sited in either ventricle and may cause obstruction of the ventricular outflow tracts resulting in fetal hydrops or intrauterine fetal death. The tumor is rounded and nonencapsulated and has a firm, white, and whorled cut surface. Histologically the tumor exhibits abundant collagen that contains uniform spindle cells. The cellularity is variable; some areas may be rather myxoid or resemble angioma. Calcification is common.

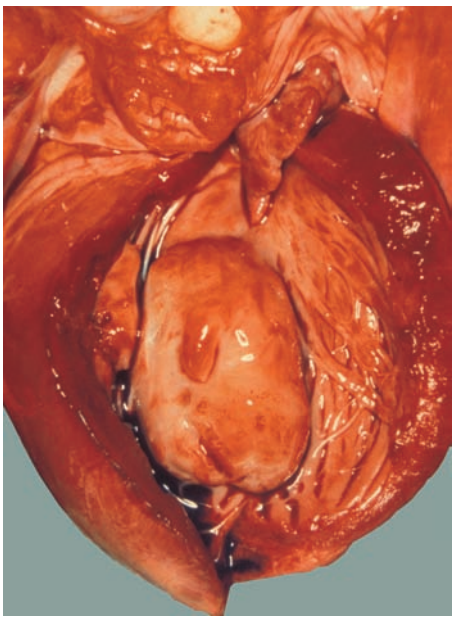


Figure 21.45. Fibroma. The left ventricle has been opened and displays a smooth mass projecting from the interventricular septum into the left ventricular cavity. The location is typical. (Courtesy of Dr. J. Keeling, Edinburgh, Scotland.)

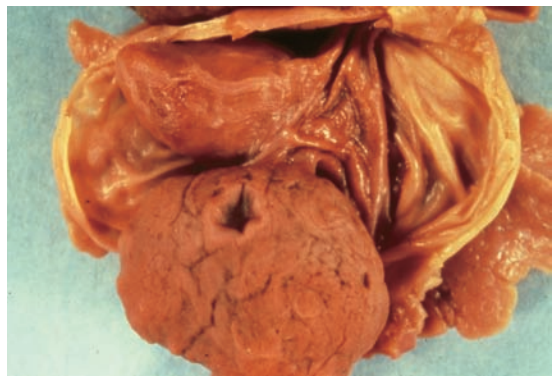


Figure 21.46. Teratoma. The pericardium has been opened and shows a large brown friable mass attached to the posterior aspect of the atrioventricular junction. (Courtesy of Dr. J. Keeling, Edinburgh, Scotland.)

Teratoma

Most teratomas of the heart occur within the pericardial cavity, usually attached to the heart near the great arteries (Fig. 21.46), but they may be intramural. The tumors are large and have a multicystic cut surface, in common with other congenital teratomas. Histologically, they consist of the usual elements found in congenital teratomas, including immature elements (Heerema-McKenney et al. 2005). Yolk-sac elements may be present. Presentation is with cardiac failure because of the compressive effect of the tumor. There may be pericardial effusion with cardiac tamponade.

Other Tumors

Tumors other than rhabdomyoma, fibroma, and teratoma are extremely rare. Myxoma, hemangioma, and mesothelioma have all been reported.

Myxoma may occur in association with the Carney complex (Amano et al. 2003).

Vascular System

Iatrogenic Disease

The intensive and invasive procedures used in neonatal care may cause injury to the vascular system (Keeling 1981). This usually manifests as mural thrombosis, sometimes with luminal occlusion occurring in the aorta or iliac arteries subsequent to umbilical arterial catheterization (Fig. 21.47) in the portal veins because of umbilical venous catheterization, or in the superior or inferior caval veins because of venous cannulation. The lesions tend to organize, leaving raised, firm, calcified mural plaques. The vessels may also be ruptured, giving rise to dissecting aneurysms or hematomas.

Marfan Syndrome

Marfan syndrome is an autosomal dominant heritable disorder of fibrous connective tissue due to mutation in the fibrillin-1 gene, located on chromosome 15. Classically, it presents with musculoskeletal abnormalities and ectopia lentis. Cardiovascular manifestations include aortic root dilatation with aortic and mitral regurgitation and dissecting aortic aneurysm. There is severe medial cystic change and degeneration in the aortic wall. Neonatal Marfan syndrome presents with incompetence of all cardiac valves with subsequent congestive heart failure and carries a particularly poor prognosis (ter Heide et al. 2005) (Fig. 21.48).

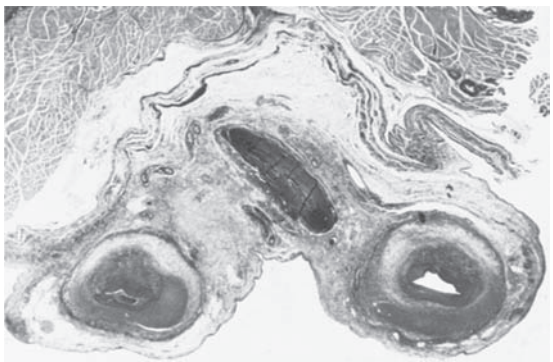


Figure 21.47. Thrombosis of both umbilical arteries related to intimal traumatic injury following catheterization.

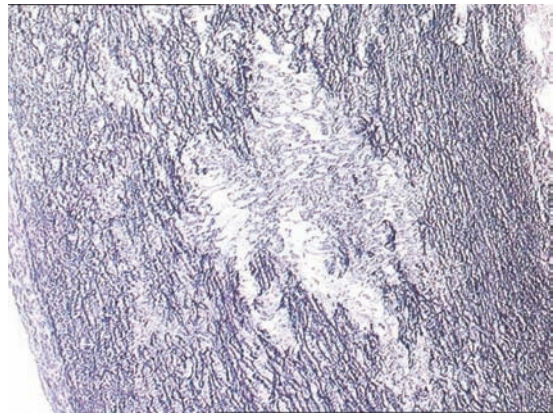


Figure 21.48. Marfan's syndrome. A section of the aorta stained with elastic van Gieson. There is interruption of the normal parallel arrangement of the elastic fibers, with their destruction and replacement by myxoid material. Fibrosis and vascularization may develop.

Fibromuscular Dysplasia

Arterial fibromuscular dysplasia is a disorder in which there is disorganization of the elements of the vascular wall with irregular thinning and thickening of the wall and encroachment on the lumen (Luschner et al. 1987). It most commonly affects the renal arteries resulting in systemic hypertension. It can occur in other sites, sometimes without renal involvement (Price and Vawter 1972). The central nervous system is a common site. Cases have been described of isolated fibromuscular dysplasia of the coronary arteries, both in adults and in children, and some of the cases are associated with sudden death (Arey and Segal 1987; Imamura et al. 1997). The epicardial or intramural arteries may be affected, and involvement of the artery to the sinoatrial or atrioventricular node is documented (Fig. 21.49).

It is likely that fibromuscular dysplasia is not a single entity but rather a phenotype indicating a limited range of responses of the arterial wall to injury. Even in the normal heart, the intramural arteries located in the papillary muscles of the atrioventricular valves may show minor dysplas-

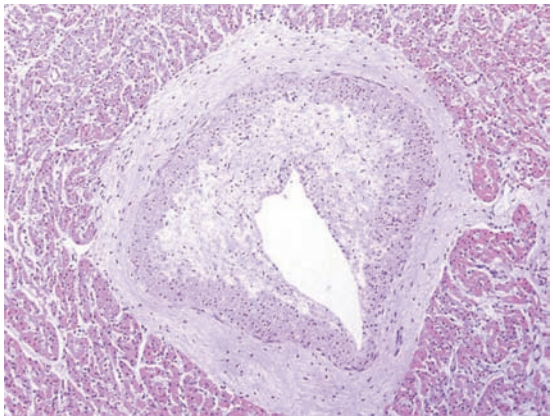


Figure 21.49. Fibromuscular dysplasia of the coronary arteries. An intramyocardial artery exhibiting irregular expansion of the adventitial, media, and intima.

tic features (Billingham 1992). Dysplastic changes are well described in the intramural coronary arteries in hypertrophic cardiomyopathy (Maron et al. 1986) and also occur, again in association with myofiber disarray, in the rare hamartoma of mature cardiac myocytes (Burke et al. 1998). Similar histopathological changes in arteries are described in congenital rubella infection (Fortuin et al. 1971), neurofibromatosis (Greene et al. 1974), and tuberose sclerosis (Rolfes et al. 1985). In areas of scarring in the ventricular myocardium the small coronary arteries will show some irregular mural thickening, probably as a secondary response.

Idiopathic Arterial Calcification

The condition is rare and it can present in utero with fetal hydrops (see Fig. 14.19 in Chapter 14) or stillbirth. There is focal or diffuse arterial calcification of the aorta, its branches, coronary arteries, and pulmonary arteries (Morton 1978). There is deposition of calcium in the elastic laminae and between them (Fig. 21.50). There is associated fibrous intimal proliferation, which may be quite striking. The disorder is usually fatal in the first few months of life because of myocardial ischemia caused by coronary artery involvement. Refractory hypertension is usually present. There may be associated pulmonary hypertension because of pulmonary arterial involvement (Farquhar et al. 2005). There is evidence of an autosomal recessive mode of inheritance, and mutations have been described in the *ENPP1* gene that encodes for a cell surface enzyme that generates inorganic pyrophosphate, a solute that regulates cell differentiation and serves as a physiologic inhibitor of calcification (Rutsch et al. 2003).

The Coronary Arteries

In the newborn, small areas of intimal fibrous or fibroelastic thickening may be observed in the coronary arteries, usually in association with arterial branching. These areas are usually associated with breaks in the underlying internal elastic lamina. Such areas of intimal thickening may also be seen in the first few millimeters of the coronary arteries after their origin from the aorta. The exact nature of these histological lesions is still debated. Some claim them to be progenitors of atherosclerosis in later life (Pesonen 2004), and others suggest that they arise at points of weakness in the vessel wall associated with branching (DeSa 2002). Similar changes may sometimes be seen in late stillbirths.

The coronary arteries are affected by idiopathic arterial calcification and fibromuscular dysplasia, both discussed above. They are also the seat of marked mural and intimal thickening in cases of supravalvar aortic stenosis and in association with the ventriculocoronary artery sinusoids in pulmonary atresia with intact septum.

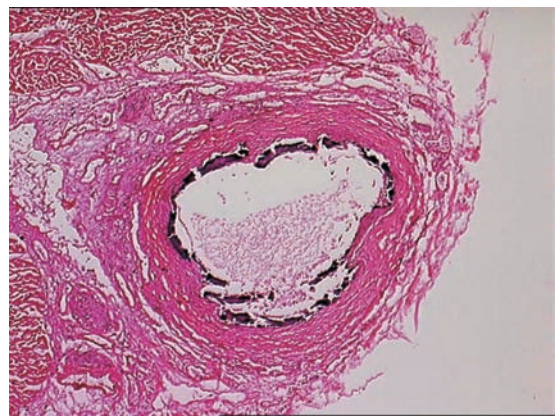
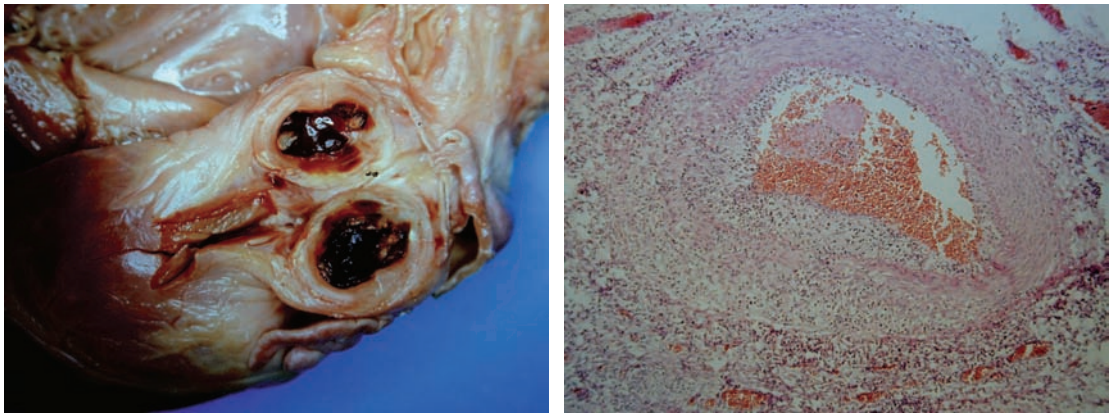


Figure 21.50. Idiopathic arterial calcification. An autolyzed epicardial coronary artery showing a thick concentric layer of intimal calcification. Similar changes were present in the lungs.



A B

Figure 21.51. (A) Kawasaki disease. Aneurysm with thrombosis in the left coronary artery. Death occurred suddenly some weeks after the original illness. (B) A section of one of the epicardial coronary arteries from the same case showing a florid, transmural arteritis. (Courtesy of Dr. H. Porter, Leicester, England.)

Abnormalities of the origin and the course of the coronary arteries are discussed above. The coronary arteries are also the site of emboli.

Kawasaki disease (Fig. 21.51) is marked clinically by fever, conjunctivitis, erythema of the mucosa of the lips and mouth, strawberry tongue, palmar erythema and desquamation, polymorphous nonvesicular body rash, and cervical lymph node enlargement. It can occur in infants younger than 6 months of age. Silent cardiac involvement is common with myocarditis and arteritis of the coronary arteries. Aneurysms develop in up to a quarter of untreated patients in the healing phase and may thrombose, causing myocardial ischemia or sudden death. In the United States, Kawasaki disease has overtaken rheumatic fever as the leading cause of acquired heart disease in children (Newburger and Fulton 2004).

The Cardiac Conduction System

The cardiac conduction system has gained an undeserved reputation as an extremely complex area of study. In fact, the anatomical makeup of the conduction system is very straightforward, and examination of it is no more difficult than, say, examination of the posterior urethra. The examination is basically a histological one and all that is required is a simple knowledge of the normal anatomy, and a little care in dissection and sampling for histology. The number of pathological processes affecting the conduction system is very limited, and minor abnormalities are unlikely to be of significance. As a cause of sudden death, conduction system abnormalities will not be subtle. More care is required in trying to correlate an abnormal electrocardiogram with postmortem findings, but one is seldom called upon to do so; the most usual situation is to be presented with a sudden and unexpected death where this question is posed: Was death due to an abnormality of the conduction system?

Normal Anatomy

There are two main components to the human conduction system: the sinoatrial node and the atrioventricular conduction axis. There is no convincing evidence in humans that specialized conduction pathways exist in the right atrium linking the two.

The sinoatrial node lies on the epicardial surface of the heart at the junction of the superior caval vein with the crest of the right atrial appendage, sometimes in front of the junction, sometimes behind, and at times astride it. Sampling of the junction includes the sinoatrial node. It must be stressed that, if it is intended to sample the node for histology, the superior cavoatrial junction should not be cut when opening the heart or the normal anatomical relations will be lost and the

sampling may not include the node. Histologically, the node comprises a triangular area of myocardium, the constituent cells of which are smaller than the surrounding "working" atrial myocardium. A very helpful marker of its site is the central large sinoatrial nodal artery (Anderson et al. 1981b).

The atrioventricular node is also roughly triangular in shape and sits at the lower part of the interatrial septum in the triangle of Koch. The borders of this triangle are the atrioventricular junction, the mouth of the coronary sinus, and the tendon of Todaro, a fibrous subendocardial prolongation of the eustachian valve that inserts into the membranous septum. The nonbranching bundle of His emerges from the node, penetrates the membranous septum, and divides atop the crest of the muscular interventricular septum to give a fan-shaped left bundle branch and a rather more discrete right bundle branch. The left bundle branch lies, for the most part, immediately beneath the endocardium of the left ventricular outflow tract; by contrast, the right bundle branch dives into the myocardium of the right side of the interventricular septum and travels towards the apex in that location (James 1970) (Fig. 21.52).

The disposition of the atrioventricular conduction tissue in the various forms of congenital heart disease has been well mapped, and in any given condition, except the most complex, the expected site of the conduction tissue can be worked out with some accuracy. Details of these patterns are beyond the scope of this work, but two examples will suffice to prove the point.

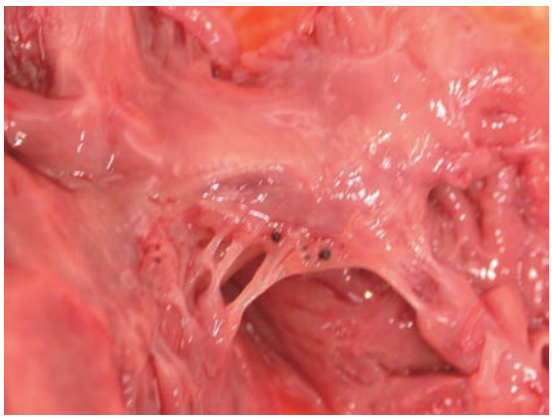


Figure 21.52. Blood cyst anterior leaflet of the mitral valve. The location at the apposing margin of the valve rather than along the free edge is typical. Similar cysts can also be found on the arterial valves.

With a perimembranous septal defect the membranous septum, within which the bundle of His travels and divides, is deficient and the bundle must reach the ventricular myocardium by traveling along the posterior and inferior edge of the defect along the exposed crest of the muscular interventricular septum where it is peculiarly vulnerable to injury during surgical repair.

In an atrioventricular septal defect, the atrioventricular septum and membranous septum are absent. The triangle of Koch is still present but is a long way from the site where the bundle of His must gain access to the ventricular myocardium. In this situation the AV node is not located in the triangle of Koch but is inferiorly place in a so-called nodal triangle inferior to the mouth of the coronary sinus. From here the bundle of His travels inferiorly to reach the crest of the interventricular septum where it divides. The bundle of His, therefore in AVSD has an abnormally long course (Feldt et al. 1970).

Histological Sampling

The sinoatrial node is sampled by taking the superior caval junction with the right atrial appendage. It can be embedded either horizontally or vertically and sectioned until the node appears.

The AV conduction axis is sampled by taking the opened right heart and making a vertical cut at the mouth of the coronary sinus and a parallel cut just lateral to the membranous septum (in effect lateral to the medial papillary muscle of the tricuspid valve). The atrium is cut by a horizontal cut about 1 cm above the AV junction and the ventricular septum is cut about 1 cm below the junction. This block will contain the AV node, the unbranched bundle of His, and both bundle branches. The block can be divided vertically, depending on its size in a plane perpendicular to the interventricular septum. The normal fetal and neonatal bundle of His shows arcs of conduction tissue extending into the membranous septum, and looping back to the node's so-called dispersion of the tissue or sometimes referred to as archipelagos. These arcs should not be taken as evidence of aberrant conduction pathways.

The most severe pathological changes are seen in infants of women with systemic lupus erythematosus (SLE) who have anti-rho antibodies. The fetus suffers congenital heart block and may have cardiac failure and hydrops. There is severe destruction with fibrosis and calcification, not just of the conduction tissue, but also of much of the atrioventricular junction and the surrounding myocardium (Ho et al. 1986).

Ischemic necrosis of the superior part of the interventricular septum can affect the AV conduction system (Fig. 21.53). In cases of viral myocarditis, if the AV tissues are sampled, inflammation, with or without necrosis, may be demonstrated in the AV node or in the bundle (Fig. 21.54) or its branches, and may contribute to sudden death in these cases (Lev and Bharati 1974). The conduction system tissues are involved in histiocytoid cardiomyopathy (Shehata et al. 1998).

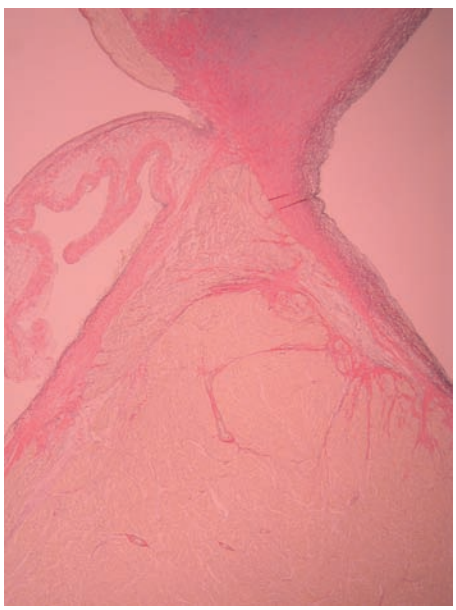


Figure 21.53. Normal atrioventricular conduction system. A section through the membranous septum stained with elastic van Gieson. The tricuspid valve is on the left of the image and the left ventricular outflow tract on the right. The bundle of His giving rise to left and right bundle branches is visible as an inverted V-shaped structure astride the crest of the muscular interventricular septum. Note its close relationship to the septal attachment of the tricuspid valve and the immediately subendocardial location of the bundle and its branches.

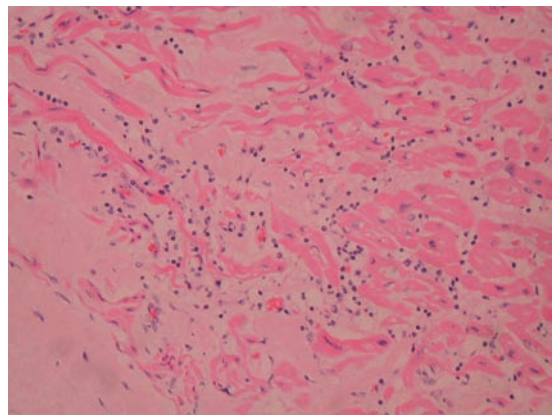


Figure 21.54. Atrioventricular (AV) node inflammation. A case of viral myocarditis. The section is taken from the bundle of His and shows edema and an inflammatory infiltrate among the myofibers without myofiber necrosis.

Arrhythmia in the Absence of Structural Heart Disease

There exists a group of children with severe, life-threatening arrhythmia considered to have occurred on a solely "electrical" basis without associated anatomical abnormality (Wever and Robles de Medina 2004). Several conditions are recognized with distinct electrophysiological abnormalities, including Wolff-Parkinson-White syndrome, long QT syndrome, the Brugada syndrome (Antzelevitch 2003), short-coupled torsade de pointes, and catecholamine-induced polymorphic ventricular tachyarrhythmia (Francis et al. 2005). The remaining patients without such distinct abnormalities are categorized as having idiopathic ventricular fibrillation. Long QT syndrome is a heterogeneous group of ion channel defects causing prolongation of myocyte repolarization, with a QT interval on the electrocardiogram corrected for heart rate, and leading to torsade de pointes ventricular tachycardia. Brugada syndrome, a familial disorder of transmembrane ion transport, is felt to be the result of a group of sodium channel defects leading to characteristic electrocardiographic abnormalities, and syncope and sudden death.

It is unquestionable that some of these patients present as sudden infant death syndrome, but in the absence of electrocardiographic or genetic evidence, the contribution, whether negligible or

Table 21.4. Cardiovascular causes of sudden infant death

Structural
Hypoplastic left heart
Transposition of the great arteries
Pulmonary atresia with intact septum
Critical aortic stenosis
Total anomalous pulmonary venous connection
Tetralogy of Fallot
Myocardial
Cardiomyopathy, hypertrophic, dilated, restrictive.
Noncompaction of the left ventricular myocardium
Histiocytoid cardiomyopathy
Rhabdomyoma
Fibroma
Coronary artery abnormalities
Anomalous origin of left coronary artery from the pulmonary trunk
Kawasaki disease
Fibromuscular dysplasia
Idiopathic arterial calcification
Infective
Viral myocarditis
Metabolic
Congenital disorders of glycosylation
Carnitine and related deficiencies
Conduction system disturbance
Long QT syndrome
Brugada syndrome
Congenital heart block
Miscellaneous

major, that these conditions make to sudden infant death is unquantifiable. One has to accept the fact that frequently, one will see nothing abnormal, even in cases where there is known electrocardiographic abnormality, such as in the long QT syndrome. Causes of sudden cardiac death are listed in Table 21.4.

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22
The Urinary System

T. Yee Khong and Adrian K. Charles

In the perinatal period, congenital abnormalities of the kidneys and urinary tract are of major importance while specific acquired lesions and tumors are comparatively rare. Acquired problems associated with prematurity, such as acute tubular necrosis, cortical necrosis, and nephrocalcinosis, are also quite common. The recognition that anomalies of the urogenital tract are often associated with other anomalies of the urogenital tract has led to the term CAKUT—congenital anomalies in the kidney and urinary tract (Nakai et al. 2003; Rodriguez 2004). Many congenital abnormalities are better appreciated by an understanding of normal renal development, and the pathophysiology of many of these anomalies are being identified at the molecular level (Nakanishi and Yoshikawa 2003). For more detailed information, the reader is advised to also consult recent embryology texts and reviews (Glassberg 2002; Levin and Schlussel 2003; Bouchard 2004). The increasingly sophisticated images obtained by the antenatal ultrasound screening program has increased the antenatal detection of renal abnormalities, allowing better management of these patients and showing the development and changing features of these diseases during gestation (Bhide et al. 2005). Tumors of the kidney in the neonate are covered in Chapter 15.

Development

In vertebrates, including humans, the development of the urinary (excretory) and genital (reproductive) systems are closely linked. Indeed, in the male embryo part of the system initially concerned with urinary excretion later becomes connected with the testis and forms part of the genital ducts; even in postnatal life the urethra forms a common channel for the urinary and genital systems. In the female embryo the primitive excretory ducts largely regress, and the definitive reproductive tract develops from a pair of paramesonephric (müllerian) ducts, wholly separate from the excretory system.

In the human embryo the kidney develops from an intermediate mass of mesoderm, the nephrogenic cord, between the lateral plate mesoderm and dorsal somites behind the intraembryonic coelom. The appearance of the definitive kidney, the metanephros, is preceded by two transient excretory organs—the pronephros and mesonephros. The pronephros develops in the most cranial, cervicodorsal segments of the nephrogenic cord. In the human embryo only a few tubules differentiate and rapidly degenerate. The mesonephros develops in the intermediate, dorsolumbar segments of the nephrogenic cord, differentiating after the pronephros but before it completely disappears. A series of vesicles that form glomeruli and tubules develop, and these join an excretory channel, the mesonephric or wolffian duct, which gradually extends to join the cloaca at the 27- to 28-somite stage (4th postconceptional week). Degeneration of the most cranial mesonephric nephrons commences before the caudal nephrons develop. The metanephros is derived into two parts: the renal parenchyma from the caudal, initially sacral, part of the nephrogenic cord; and the excretory system (the

collecting tubules, calyces, pelvis, and ureter) from the ureteric bud. The latter forms as a posteromedial outgrowth from the wolffian (mesonephric) duct near its junction with the cloaca when the embryo measures around 5 mm. Its proximal end extends dorsally to impinge on the metanephric blastema, while its distal end grows caudally with growth of the embryo.

The ureteric bud develops a dilated terminal portion, the ampulla, and on contact with the metanephric blastema this undergoes a series of dichotomous branching, each branch developing a separate ampulla at its tip. The genes *GDNF* and *ret* are crucial for this development. The caudal unbranched portion of the ureteric bud forms the ureter. The first few generations of branches coalesce to form the renal pelvis and calyces, while subsequent divisions form the collecting ducts. From the end of the 7th week (18- to 20-mm stage), formation of the collecting system is accompanied by nephrogenesis. Oval condensations of metanephric blastemal cells become related to each ampulla. Some of these cells form a blastemal cap, which accompanies the next subdivision of the ampulla, while others form a hollow vesicle, which elongates and becomes S-shaped. The lumen becomes continuous with that of the related ureteric bud branch, and at the other end capillary vessels develop in situ to form the glomerulus. The intervening portions develop into the proximal and distal convoluted tubules with the loop of Henle in the middle. Nephron attachment to the ampullae varies at different stages of organogenesis Osathanondh and Potter 1963a,b, 1964a–f, 1966). After the initial phase (7 to 14 weeks), branching of the ureteric bud ceases, and each ampulla becomes capable of inducing formation of a number of nephrons (14 to 22 weeks), which are joined together in a chain (nephron arcade). After the 22nd week, nephrons in the outer cortex become attached singly behind the ampulla until new nephron formation stops at between the 32nd and 36th weeks. These last-forming nephrons can be recognized in the subcapsular cortex in sections of fetal kidneys of less than 36 weeks' gestation as a "nephrogenic zone," a reliable histological marker of prematurity. Until that time the kidney is of great interest because successive generations of glomerular development are present, allowing an appreciation of all stages of nephrogenesis to be observed in a single case. During its early development the metanephros is at the level of the upper sacral segments and it receives its blood supply from lateral sacral branches of the aorta. As the embryo increases in length, the relative position of the developing kidney becomes progressively more cranial until by the 8th week it is in its definitive lumbar situation. The "ascent" is accompanied by medial rotation so that the renal pelvis, which is initially ventrally placed, comes to lie on the medial aspect of the kidney. During its ascent the kidney receives blood from stem arteries from the aorta at progressively higher levels until ultimately it is supplied by the definitive renal artery at the level of the 2nd lumbar vertebra.

The cloaca, marked externally by a shallow depression covered by the cloacal membrane, is continuous with three endodermal tubules: the hindgut, the allantois, and the blind-ending diverticulum of the tail gut. At about the 5-mm stage a septum, the urogenital septum, extends down caudally from the angle between the allantois and the hindgut and ultimately fuses with the cloacal membrane at the 16-mm stage, thus dividing the cloaca into a smaller dorsal portion, the primitive rectum, and a larger ventral part, the primitive urogenital sinus. The terminal portions of the wolffian (mesonephric) ducts beyond the origin of the metanephric ducts, now termed the common excretory ducts, join the urogenital sinus on the two sides, dividing it into an upper portion above the ducts—the vesicourethral canal—and a lower portion below the ducts—the definitive urogenital sinus.

The vesicourethral canal, from which the bladder and upper urethra develop, is a slightly flattened cylinder, continuous cranially with the allantois. As the lower abdominal wall grows, the bladder segment of the vesicourethral canal enlarges and the urogenital sinus below it becomes flattened from side to side and elongates dorsoventrally. The common excretory ducts dilate and then become absorbed in the wall of the vesicourethral canal so that the metanephric and mesonephric ducts drain separately into the vesicourethral canal. As the bladder enlarges, the metanephric (now ureteric) orifices move gradually caudally and laterally in relation to the mesonephric ducts, which remain close together near

the midline. In the male embryo the mesonephric ducts persist to form the epididymis, vas deferens, seminal vesicle, and common ejaculatory duct. In the female the mesonephric duct degenerates from about the 30-mm stage and ultimately disappears, apart from the vestigial epoophoron, paroophoron, and Gartner's duct. The triangular zone and common ejaculatory duct between the ureteric orifices form the trigone of the bladder, which is of mesodermal origin, unlike the rest of the bladder, which is derived from endoderm. The allantois regresses as the bladder enlarges and ultimately forms a solid fibrous cord, the urachus, extending from the bladder to the umbilicus.

In the female, the primitive urethra, derived from the lower part of the vesicourethral canal, forms almost the whole of the definitive urethra. In the male this forms only the upper portion of the prostatic urethra and the membranous urethra is derived from the upper (pelvic) part of the definitive urogenital sinus, while the penile urethra is formed by the fusion of two urethral folds, which form on each side of the ventral surface of the developing phallus. The urethral groove, which lies between the two folds, is formed by a plate of cells (the urethral plate), which extends forward from the lower (phallic) portion of the urogenital sinus. Fusion of the urethral folds starts posteriorly and extends forward; it is marked posteriorly by the median raphe. Fusion extends forward only as far as the circular coronary sulcus, which delineates the glans penis. A core of ectodermal cells grows backward through the glans to join with the urethra. This later becomes canalized and is continuous with the urethral lumen, thus forming the terminal (glandular) part and the urethral meatus.

Genetic Regulation

Various gene families and their products can be demonstrated in the developing mammalian metanephros by in-situ hybridization (ISH) or by immunohistochemical techniques, respectively. These include transcription factors, growth factors, and adhesion molecules. The spatial and temporal nature of their expression and their ablation in transgenic mouse models allows speculation about their role in normal renal development, as well as mechanisms whereby genetic aberrations may be involved in developmental abnormalities like renal agenesis, dysplasia, polycystic disease, and embryonal neoplasms such as nephroblastoma. Our understanding of the processes involved in renal development is rapidly progressing (Glassberg 2002; Bouchard 2004). The reader is referred to other sources detailing the latest genetic information (Davies 2006), as this chapter cannot review the topic in depth because of space limitations. Recent progress in genetics has led to a clearer understanding of the pathophysiology of the diseases, for example, in the nephrotic syndromes described below.

Transcription factors regulate the expression of other genes by enhancing or switching off messenger RNA (mRNA) transcription. Transcription factors expressed during nephrogenesis include products of various homeobox (*hox* and *pax*) genes, the *WT1* Wilms' tumor suppressor gene, and the *myc* family of genes. Homeobox genes control regional and positional specification in the early embryo. In-situ hybridization studies have identified specific patterns of expression for *pax2* and *pax8* genes in the developing murine kidney (Dressler et al. 1990; Plachov et al. 1990), and similar expression of *pax8* has been demonstrated in the human fetal kidney (Eccles et al. 1992). These genes are located in the nuclei of pro- and metanephric duct epithelial cells, and in the caps of condensed metanephric blastema associated with the ampullae of ureteric bud branches. When S-shaped bodies develop from metanephric vesicles, *pax2* expression is quickly downregulated, and it is absent from mature nephrons, suggesting involvement of the gene product in the induction of metanephric blastema to fully developed tubular epithelium. Pax-2 has a role in mesenchymal to epithelial differentiation and also is increased in cystic renal disease.

The *WT1* Wilms' tumor suppressor gene located on chromosome 11p13 is expressed at the inception of the metanephros (Pritchard-Jones et al. 1990; Kreidberg et al. 1993; Larsson et al. 1995). Its expression increases in the nephrogenic vesicles, particularly in the podocyte epithelial layer of the glomerulus when it first forms, but its expression signal soon decreases and disappears, so that in sections of the fetal kidney it is

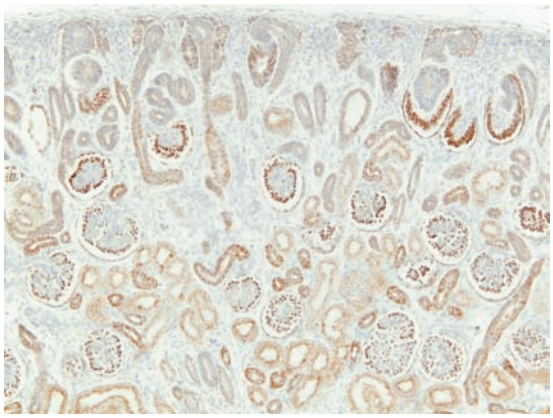

Figure 22.1. *WT1* staining of fetal kidney at 18 weeks showing staining of nephrogenic zone and blastemal elements.

confined to the subcapsular nephrogenic zone (Pritchard-Jones et al. 1990) (Fig. 22.1). The protein encoded by *WT1* binds to several sites on promoters of an insulin-like growth factor-2 (IGF-2) gene (Drummond et al. 1992) and to a promoter of the platelet-derived growth factor A chain (PDGF-A) gene (Gashler et al. 1992). *WT1*, therefore, may inhibit IGF-2 and PDGF-A production in the developing kidney, thereby inhibiting cellular proliferation and initiating cell differentiation. Interference with these regulatory functions of *WT1* may be relevant in the development of nephroblastoma, in which high levels of IGF-2 are expressed, and there is overproduction of PDGF (Scott et al. 1985; Fraizer et al. 1987). If *WT1* is ablated by null mutation in a murine model, no kidneys are formed.

Glial-derived neurotrophic factor (GDNF) produced by the mesenchyme interacts with the c-*ret* proto-oncogene receptor on the ureteric bud and plays a crucial role in the branching pattern of the developing uteric bud (Shakya et al. 2005). Many other genes including *PAX2* and *WT1* are also important in this branching process, and mouse knockout models have demonstrated the range of renal anomalies associated with gene defects (Piscione and Rosenblum 2002).

Growth factors bind to cell surface receptors, particularly receptor tyrosine kinases. After ligand binding these receptors dimerize and become autophosphorylated, after which they can transduce signals into the cell. Growth factors not only cause cell division, but may also stimulate cell survival, differentiation, morphogenesis, and apoptosis. The factors of this type expressed in the developing kidney include fibroblast growth factors (FGFs), hepatocyte growth factor/scatter factor (HGF/SF), insulin-like growth factors 1 and 2 (IGF-1 and -2), neurotrophins (neurotrophin 3 and nerve growth factor, NGF), PDGF A chain and B chain, transforming growth factor-α (TGF-α), and vascular endothelial growth factor (VEGF).

There are two types of adhesion molecules—those mediating cell-to-cell attachment, and those mediating cell-to-surrounding-matrix attachment. Examples of cell-cell adhesion molecules expressed during nephrogenesis include neural cell adhesion molecule (NCAM) and E-cadherin (uvomorulin), and examples of cell-matrix adhesion molecules include collagens, fibronectin, galectin-3, laminins, nidogen, and tenascin. These not only bind to plasma membranes by intergrin receptors, but also interact with each other. The KAL protein is a putative adhesion molecule on the basis of its homologies with NCAM and fibronectin. The *KAL* gene is mutated in X-linked Kallmann syndrome, which is characterized by developmental anomalies of renal and olfactory bulb development.

Proto-oncogenes encode various cellular proteins, such as growth factors, growth factor receptors, intracellular transducers, and nuclear transcription factors that are involved in normal cell growth, proliferation, and differentiation. The *myc* family and c-*ret* genes are important in the developing kidney. The *myc* family genes show distinctive but contrasting expression in the metanephros; c-*myc* is seen in uninduced metanephric blastema, in S-shaped bodies, and in nephronic tubules, while N-*myc* is found exclusively in condensations of metanephric blastema related to ureteric bud ampullae. N-*myc* expression increases as mesenchymal cells differentiate into epithelial cells, and is then rapidly down-graded as epithelial polarity develops.

The c-*ret* gene encodes a tyrosine kinase receptor and is expressed in ureteric bud epithelium as well as in the developing central and enteric autonomic nervous systems. Mice homozygous for a targeted mutation in c-*ret* die soon after birth and exhibit enteric aganglionosis and urinary tract anomalies ranging from complete ureteric and renal agenesis, through blind-ending ureters with

renal agenesis, to complete ureters with small dysplastic kidneys.

Malformations of the Kidney

Congenital renal abnormalities can be divided into (1) anomalies of position and form and (2) parenchymal maldevelopments.

Ectopia and Malrotation

Normal "ascent" and rotation of the kidney during development has been described above. Interference with, or arrest of, this process results in the permanent abnormal position of the kidney, which is often rounded or lobulated rather than reniform because of the different molding effects of adjacent structures. It is also more commonly malrotated, with the renal pelvis pointing forward instead of medially. Rarely, reversed rotation, or rotation through more than 90 degrees results in a laterally or posteriorly directed renal pelvis. Most commonly, ectopic kidneys are located at the pelvic brim or in the pelvis. Often, the ectopic kidney is supplied by a number of small arteries rather than a single vessel; these arise from the aorta near its bifurcation or from the iliac arteries. Not uncommonly, ectopic kidneys exhibit renal dysplasia (see p. 629), and sometimes distortion or kinking of the renal pelvis or ureter may lead to hydronephrosis and predispose to renal infection and calculus formation. Occasionally, the ureter of an ectopic kidney is inserted ectopically in the bladder, vagina, or seminal vesicle; sometimes it is subject to vesicoureteric reflux. Rarely, ectopic kidneys are located at a higher than normal level, even in the thorax in association with a diaphragmatic defect. High ectopia is almost always on the left side and is virtually confined to males.

Renal ectopia may be unilateral or bilateral and has an incidence of about 1 in 800. It is slightly commoner in females and on the left side. Rarely, unilateral renal ectopia is associated with absence (agenesis) of the contralateral kidney, and pelvic ectopia may accompany anorectal anomalies or absence of the vagina in females. Around 14% of cases are associated with other anomalies of the urogenital tract (Guarino et al. 2004).

Ectopic renal tissue may be found in the spinal canal (in "lipomas") usually associated with neural tube defects (Horenstein et al. 2004) and adjacent to the gonads in fetuses, where it probably represents persisting mesonephric glomeruli. The extrarenal locations of nephrogenic tissue may be the explanation of extrarenal Wilms' tumors. Renal tissue may be seen in sacrococcygeal teratomas. Occasionally in sirenomelia and rarely in other conditions, renal tissue may be seen within the wall of the distal gut (Jain et al. 2002).

Crossed Ectopia

Crossed ectopia is a rare anomaly (with an incidence of about 1 in 8000) that is characterized by the presence of an ectopic kidney on the opposite side from the ureteric orifice, so that the ureter crosses the midline and both kidneys are situated on the same side of the body, the ectopic kidney lying below the orthotopic kidney. Almost always the two kidneys are fused. Dysplastic development of the crossed kidney is sometimes seen and the ureter may be dilated and subject to vesicoureteric reflux.

Fusion

The commonest variety is where the lower poles of the two kidneys fuse across the midline (the "horseshoe" kidney), which is seen in approximately 1 in 600 radiological examinations of the kidneys. The "ring" or "doughnut" kidney, where both poles are fused, is much rarer. Since fused kidneys are usually situated lower than normal, they may also be regarded as ectopic. The renal pelves are usually anteriorly placed, and high insertion of the ureter into the pelvis at the pelviureteric junction may lead to hydronephrosis. Dysplastic development of part or the entire fused kidney may occur. Fusion is about twice as common in males. There is an elevated incidence in Turner's syndrome and in association with other congenital urogenital anomalies.

Renal Agenesis

Renal agenesis, in which the kidney and ureter are completely absent, is ascribed to a failure of development of the ureteric bud from the mesonephric

duct (Burk and Beaudoin 1977) or to its early degeneration. The absence of the ureteric bud results in a failure of the metanephric blastema to develop; the corresponding trigonal area of the bladder is also absent in the majority of cases.

Renal agenesis may be unilateral or bilateral. Unilateral agenesis has a frequency of about 1 in 1000 at necropsy (Doroshow and Abeshouse 1961), but a higher incidence based on abdominal ultrasound has been reported (Roodhooft et al. 1984); the condition is equally common in both sexes. Other congenital urogenital tract anomalies are often associated. In the male, the testis often fails to descend, and mesonephric duct derivatives such as the seminal vesicle and vas deferens are frequently absent. In the female, paramesonephric (müllerian) duct derivatives such as the fallopian tubes, uterine horn, and upper vagina fail to develop or are abnormal. Outside the urogenital tract, anomalies such as an imperforate anus, esophageal atresia, and tracheoesophageal fistula, neural tube defects, and congenital heart lesions are not infrequent.

Bilateral renal agenesis is comparatively rare (1 in 4000 births) and is twice as common in males as females. The condition is not compatible with life; death occurs in utero (for reasons that are not clear) or in the neonatal period from respiratory insufficiency. Bilateral agenesis is associated with Potter's syndrome comprising pulmonary hypoplasia, low-set ears, beak-like nose, receding chin, large hands, bow legs, and talipes (Potter 1946), which are attributed to oligohydramnios associated with this condition (Fig. 22.2). The bladder and urethra are often absent, or the urethra is atretic and the bladder minute. Bilateral renal agenesis is also associated with lower limb deformities, particularly sirenomelia (posterior limb bud fusion with absence of the sacrum, hindgut, external genitalia, urethra, and bladder). Although the kidney is the site of erythropoietin production, the fetuses tend not to be particularly anemic, though loss of other hormones such as renin may have some effects on fetal metabolism.

Bilateral pulmonary hypoplasia is almost invariable, with lung weights less than half that expected for gestational age. There are fewer than expected alveoli in relation to the number of bronchi, and there is a patchy failure in the normal process by which alveolar ducts become vascularized by mid-gestation before the alveoli develop. Aspiration of amniotic fluid is considered to be necessary for normal pulmonary development, and the pulmonary hypoplasia associated with bilateral renal agenesis is usually ascribed to oligohydramnios; monoamniotic twins discordant for renal agenesis may not have pulmonary hypoplasia (McNamara et al. 1995). The observation that amniotic fluid is largely derived from nonrenal sources during the first trimester indicates that other factors may also interfere with early pulmonary development in bilateral renal agenesis.

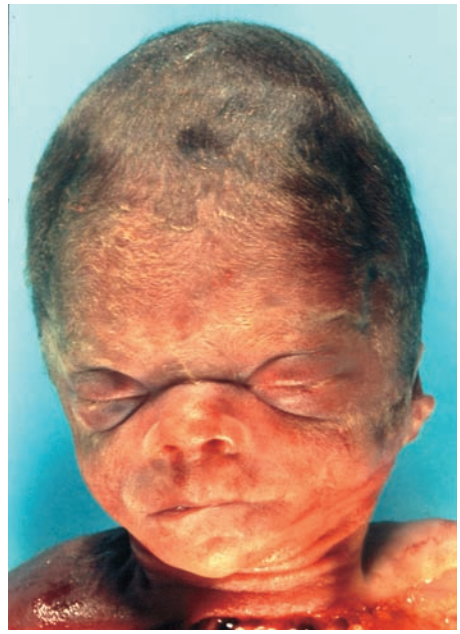

Figure 22.2. Potter's facies with furrowed eyes and nose bridge, beak-like nose. and low-set ears.

Although usually sporadic, the occasional familial incidence of bilateral renal agenesis has been recognized for some time. Unilateral and bilateral agenesis, unilateral and bilateral renal dysplasia, or dysplasia of one kidney and agenesis of the other (renal adysplasia) (Fig. 22.3) have been recorded in various combinations in the same family (so-called familial renal adysplasia or the agenesis–dysplasia syndrome) (Murugasu et al. 1991). The dysplastic kidneys in this syndrome are usually tiny and rudimentary (aplastic) or multicystic and the renal agenesis, though often accompanied by anomalies of mesonephric or

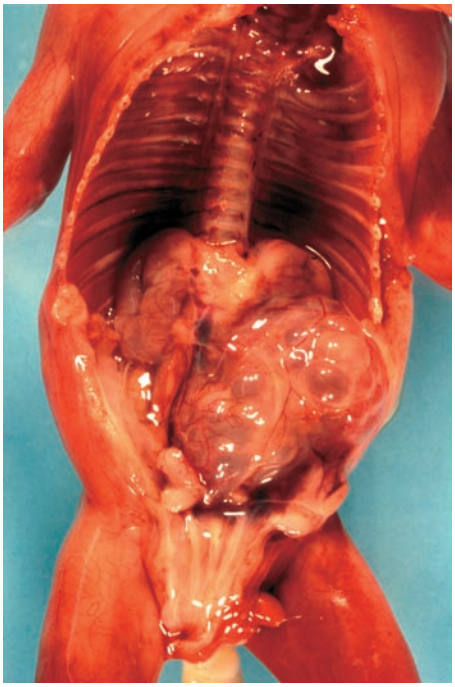

Figure 22.3. Renal adysplasia, with right renal agenesis and left renal dysplasia. The right adrenal is disk-shaped from the absence of a molding effect of the kidney.

paramesonephric duct derivatives, particularly ipsilateral ovarian or oviductal agenesis, is not associated with sirenomelia or caudal regression. Index cases usually present as perinatal deaths with bilateral renal agenesis. Family studies then reveal unilateral agenesis or dysplasia in parents or living siblings and perhaps other deaths from bilateral agenesis, dysplasia, or adysplasia in other siblings; sometimes other generations of the family are found to have been affected. Ultrasound scans sometimes document the involuting multicystic kidney. Studies indicate an autosomal dominant inheritance with variable expression (Buchta et al. 1973).

Moerman et al. (1994) described a patient with hereditary renal adysplasia who showed a de novo autosomal 6p/19q translocation with breakpoints at 6p21.3 and 19q13.4. It was suggested that 6p is the probable locus for at least one defective gene involved in hereditary adysplasia since Izquierdo et al. (1992) have indicated the importance of this site for genes involved in normal renal development following linkage studies in families with hereditary hydronephrosis. The Uroplakin IIIa gene has been implicated in some of these (Jenkins et al. 2005).

Supernumerary Kidneys

"Extra" kidneys are very rare, and their definition requires complete separation of two kidneys on one side of the body with completely separate pelvicalyceal systems. The draining ureters may fuse or join the bladder separately, or be ectopic, draining into other parts of the urogenital tract.

Renal Hypoplasia

Normally there is close correlation among body size, age, and renal weight throughout childhood. Renal hypoplasia can thus be defined as the presence of a kidney that is congenitally small (i.e., less than 2 standard deviations below the expected mean when correlated with age or the usual parameters of somatic growth). Clearly, reduction in renal size may be a result of contracture following acquired disease, but even in the neonatal period, when this is less of a problem, the definition may be hard to apply. This is because the great majority of congenitally small kidneys exhibit evidence of parenchymal maldifferentiation. Currently it is customary to label such kidneys as dysplastic and to retain the term *renal hypoplasia* for kidneys in which parenchymal differentiation is normal and that are abnormal only in terms of their small size, reduced nephron population, and perhaps reduced number of lobules (reniculi). Defined strictly in this sense, pure renal hypoplasia without dysplasia is rare, although its exact incidence is difficult to judge since in the past many dysplastic kidneys have been called hypoplastic. In practice, the frequent coexistence of other urinary anomalies such as ureteral ectopia, ureterocele, etc., is a useful pointer to a diagnosis of renal dysplasia.

Nevertheless, it is true that some ectopic or malrotated kidneys are smaller than expected, their reduced mass possibly being related to their anomalous blood supply and, in this sense, they may be regarded as hypoplastic. Such cases apart, unilateral simple hypoplasia is extremely

infrequent, and one of the few acceptable cases described is that of Bernstein and Meyer (1964). So-called segmental hypoplasia (the Ask–Upmark kidney), often associated with hypertension in older children, is now generally regarded not as a developmental abnormality but rather as a form of reflux nephropathy (Risdon 1981b).

Bilateral simple hypoplasia has been described in association with long-standing disease or congenital anomalies of the central nervous system (Roosen-Runge 1949) and Down syndrome (Mercer et al. 2004). Since the numbers of renal lobules in these cases are usually not reduced, the possibility exists that their small size reflects a failure of postnatal growth rather than an intrinsic congenital insufficiency in renal mass. In addition, there is a distinctive nonfamilial form of extreme bilateral hypoplasia termed, in French, *oligoméganéphronie* (oligonephronic hypoplasia) by Royer et al. (1962). In this condition, the kidneys are tiny and often consist of only one or two lobules. Histologically, the nephron number is drastically reduced, but those present are markedly hypertrophied. On average, the cross-sectional areas of glomeruli are increased 12-fold, and microdissection studies show that proximal tubules are increased fourfold in length and 17-fold in volume (Fetterman 1970).

Congenital Nephromegaly

Compensatory growth and hypertrophy of one kidney may occur even in utero when the contralateral kidney is severely dysplastic or absent.

Bilateral nephromegaly is a feature of the Beckwith-Wiedemann syndrome (BWS), characterized by visceromegaly, hyperinsulinemic hypoglycemia due to islet cell hypertrophy, microcephaly, exomphalos, macroglossia, and adrenal cytomegaly due to genetic changes at 11p15 (Cohen 2005; Weksberg et al. 2005). Perlman syndrome, which appears to be autosomal recessive, is characterized also by bilateral nephromegaly with nephroblastomatosis and by an abnormal facies, visceromegaly, and islet cell hyperplasia, but exomphalos and macroglossia are absent [On-Line Mendelian Inheritance in Man (OMIM) number 267000]. Cryptorchidism is present in boys in Perlman syndrome but absent in BWS.

In both these conditions the kidneys are variably enlarged and show accentuated lobulation. The medullary pyramids are poorly defined and many exhibit ductal dysplasia. Superficial nodular nephroblastomatosis (see p. 352) is frequently present, and its malignant potential for the development of nephroblastoma is reflected in the increased incidence and common bilaterality of this tumor in patients with the BWS and Perlman syndromes. Simpson-Behmel Golabi (OMIM 312870) is another overgrowth syndrome with large, often cystic kidneys, which is related to mutations of the glypican-3 gene, which is associated with the IGF-2 receptor, which may explain some of the overlap of features with BWS.

Renal Dysplasia

The term *renal dysplasia* defines a developmental abnormality of the kidney resulting from anomalous metanephric differentiation (Risdon 1971a). Almost invariably, there are other associated congenital anomalies of the ureter or lower urinary tract.

Dysplastic renal development may affect the whole kidney or it may be focal or segmental in distribution, with parts of the renal cortex often appearing to have developed normally. The pattern of dysplasia is related to the accompanying urinary tract anomalies, and this association is useful in classifying the condition (Risdon 1971a,b).

Renal dysplasia encountered in the neonatal period, and particularly in perinatal necropsies, is most commonly either of the multicystic or aplastic varieties; it may also occur in association with congenital lower urinary tract obstruction or rarely with syndromes of multiple malformations (see p. 636).

Renal dysplasia may be due to a variety of pathogenetic pathways: genetic changes directly affect renal development, which secondarily affects the lower renal tract; fetal urinary obstruction gives rise to secondary renal changes and dysplasia; and a final mechanism is genetic changes that affect the development of both the urinary tract and the kidneys (Matsell and Tarantal 2002; Shibata and Nagata 2003; Woolf et al. 2004). The interaction of fetal urinary obstruction

and genetic changes are not clear, but there is upregulation of PAX2 of the tubular epithelium with urinary obstruction and mouse models that overexpress PAX2 develop renal cysts, suggesting how renal obstruction may induce secondary genetic changes (Woolf and Winyard 2002).

Multicystic and aplastic dysplasia are forms of severe generalized corticomedullary maldifferentiation affecting the whole of one or both kidneys. The multicystic dysplastic kidney is grossly cystic and as a result is enlarged to a variable extent. The largest cysts are distributed peripherally beneath the capsule of the kidney, where they distort its reniform shape (Fig. 22.4). The pelvicalyceal system is severely attenuated or absent, reflecting the diminution in branching of the ureteric bud during organogenesis. Examination of large, correctly orientated sections usually reveals some evidence of cortocomedullary differentiation with scanty radially aligned primitive ducts, representing the diminished ureteric bud derivatives, which extend from the hilus toward the capsule. These are lined by columnar epithelium and are surrounded by mantles of mesenchymal spindle cells. Scanty immature ductal and nephronic elements are seen in the cortex, separated by loose mesenchyme. The nephron population is variably but usually severely diminished, and cyst formation is a prominent feature. The large subcapsular cysts have fibrous walls lined by flattened epithelium and are mostly derived from ureteric bud branches that have failed to induce nephron formation. Smaller cysts, representing dilated tubular, ductal and glomerular elements, are also seen. Occasionally, bars of metaplastic cartilage are present and are useful in diagnosis.

The aplastic kidney is an essentially similar malformation distinguished only by its much smaller size. It is extremely rudimentary, consisting only of a nubbin of dysplastic renal tissue or a tiny collection of cysts.

With both multicystic and aplastic kidneys, the draining ureter is usually atretic for part (usually the upper third) or all of its length or, rarely, it is completely absent.

Bilateral multicystic or aplastic renal dysplasia are lethal malformations with clinical features like those of bilateral renal agenesis; Potter's syndrome is usually present. Clinically the only distinction is that with bilateral multicystic kidneys, cystic loin masses may be palpable or may be demonstrated by ultrasonography.

Unilateral multicystic or aplastic dysplasia is usually present in the perinatal period and may be associated with major congenital anomalies in other systems, most notably isolated ventricular septal defect, aortic coarctation, intestinal atresia, and meningomyelocele. In addition, with unilateral disease there is an increased incidence of contralateral congenital ureteric stenosis and hydronephrosis.

Multicystic kidneys can be recognized in utero by fetal ultrasound, and serial examinations have shown that the size and configuration of the cysts may alter with time or even apparently disappear.

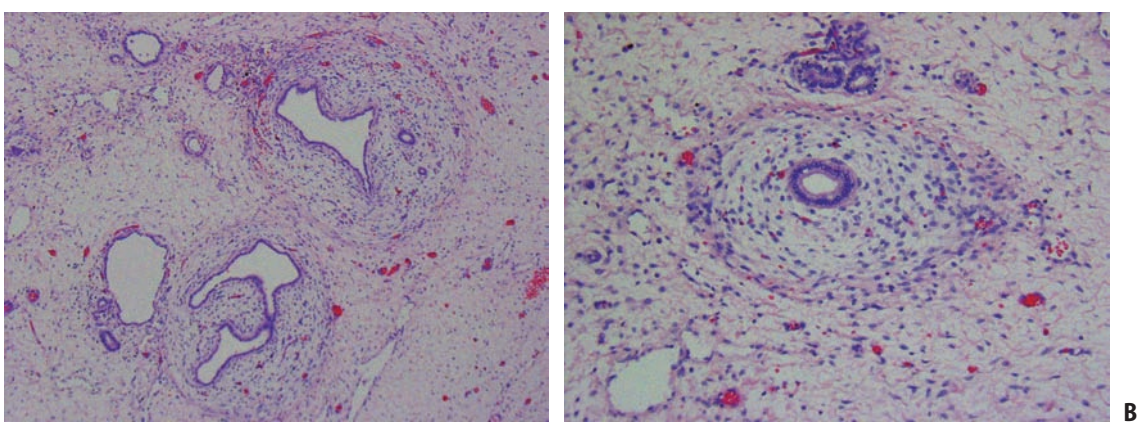

Figure 22.4. Renal cystic dysplasia showing primitive ducts and cysts lined by collarettes of mesenchyme (A,B) and metaplastic cartilage.

Since it is unlikely that a multicystic kidney will entirely regress, it is possible that the loss of the cystic ultrasound image denotes the progression of a multicystic to an aplastic kidney and is further evidence that the two conditions are intimately related.

There are many syndromes associated with renal dysplasia, with over 200 listed in the OMIM database. This reflects the numerous genes involved in renal and urinary tract development, and also that cases with renal dysplasia should be carefully reviewed to identify other dysmorphic features.

Anatomical or functional congenital lower urinary tract obstruction causes bilateral hydronephrosis and hydroureters, and there may be additional dysplastic development of the kidneys. The extent of both hydronephrosis and dysplasia is related to the degree of obstruction and also the stage of development of the insult. There is also a relationship with the severity of any accompanying vesicoureteric reflux, which can be asymmetrical. Dysplasia only on the refluxing side of patients with posterior urethral valves and unilateral vesicoureteric reflux has been described. The commonest cause of obstruction in this age group is posterior urethral valves in infant boys (see p. 644), but occasionally there is urethral atresia, which is invariably accompanied by profound renal dysplasia. Functional obstruction, occurring in the megacystic-megaureter syndrome or as part of the "prune belly" syndrome (see p. 644), may also be associated with dysplastic renal development. Animal models have also demonstrated the development of renal changes in early obstruction (Duncomb et al. 2002; Matsell and Tarantal 2002; Pringle et al. 2003). This has led to consideration of fetal surgery, but these techniques are still being established into routine care (Quintero 2005).

Whatever the cause of the obstruction, the changes in the kidney are similar and represent a combination of hydronephrosis and dysplastic differentiation, together with secondary regressive changes and pyelonephritic scarring reflecting the commonly associated ascending urinary infections suffered by these patients (Risdon 1971a,b). There is variable parenchymal thinning with flattening of the medulla associated with calyceal dilation. The flattened medulla contains a sparse system of ducts surrounded by mesenchymal connective tissue in a delta-like pattern. Cortical dysplasia is most marked peripherally beneath the capsule, affecting nephrons formed late in renal development. Glomerular and tubular elements of immature morphology are present, often separated by loose mesenchyme. Cyst formation may be prominent and occasional foci of metaplastic cartilage may also be present. Histological evidence of renal parenchymal infection may be superimposed in infants.

Occasionally ureteric atresia accompanied by profound multicystic dysplasia is seen in association with urethral atresia.

Congenital Hydronephrosis

Hydronephrosis with varying degrees of pelvicalyceal dilation, parenchymal atrophy, and blunting of medullary pyramids may develop in utero, when it is usually associated with obstruction at the pelviureteric junction. This can be variously attributed to (1) an intrinsic abnormality of the ureteric muscle bundles at this point, producing a narrow aperistaltic segment; (2) an extrinsic abnormality, such as aberrant segmental arteries crossing the pelviureteric junction, kinks, fibrous hands, or adhesions producing abnormal angulation of the ureter against the renal pelvis; or rarely (3) mucosal folds or polyps. The extrinsic causes tend to occur in older children (Dewan et al. 1998).

The condition may be unilateral or, in about 20% of cases, bilateral. The latter cases are more likely to present in infancy, and in this age group boys are two to three times more frequently affected than girls. Occasionally extreme degrees of unilateral hydronephrosis (so-called giant hydronephrosis) present as large, easily palpable, cystic loin masses in the neonatal period. Parenchymal atrophy in such cases is extreme and may be associated with dysplastic changes.

Renal Tubular Dysgenesis

Renal tubular dysgenesis, first described in 1983, is characterized by diminished to absent differentiation of the proximal convoluted tubules,

leading to oligohydramnios and Potter sequence (Allanson et al. 1983). Onset of oligohydramnios after 18 weeks' gestation in the absence of ruptured membranes and the finding of apparently normal kidneys on morphology scan and normal fetal blood flow are important antenatal clues to the diagnosis. Affected babies are generally stillborn or die shortly after birth from respiratory and renal failure. Findings associated with renal tubular dysgenesis include widely patent fontanelles, hypoplastic calvaria, and hemochromatosis (McFadden et al. 1997; Morris et al. 2004). Renal tubular dysgenesis has been described in association with trisomy 21 (Jain and Beneck 2003).

Renal tubular dysgenesis affects both male and female siblings, and consanguinity has been reported, suggesting that inheritance is autosomal recessive (Allanson et al. 1992). Restricted differentiation of primitive tubules due to renal hypoperfusion is a likely cause in many cases, especially those seen in twin-to-twin transfusion syndrome and following maternal administration of angiotensin-converting enzyme (ACE) inhibitors and indomethacin (Cunniff et al. 1990; Barr and Cohen 1991). Mutations of genes of the renin angiotensin system are found in the autosomal recessive condition (Gribouval et al. 2005). The association with neonatal hemochromatosis may reflect a fetal form of the hepatorenal syndrome, which may also explain the oligohydramnios often seen in this condition.

Grossly, the kidneys are usually of normal shape and size, although the affected kidneys may be moderately enlarged. On microscopical examination the glomeruli appear abnormally crowded together, and the tubules, which are lined by small darkly staining cells, cannot be differentiated histologically into proximal and distal portions. The characteristic periodic acid-Schiff (PAS)-positive brush border of proximal tubular cells is absent. Immunohistochemical staining with epithelial membrane antigen or peanut lectin shows positive staining of all tubular cells (normally, only distal tubules are stained), while staining for lysozyme, which typically labels proximal tubules, is absent (Moldavsky 2000) (Fig. 22.5). Immunostaining for renin is marked increased in preglomerular arterioles, glomerular hilum, and mesangium, suggesting a pathogenic role for the renin–angiotensin system (Bernstein and Barajas 1994).

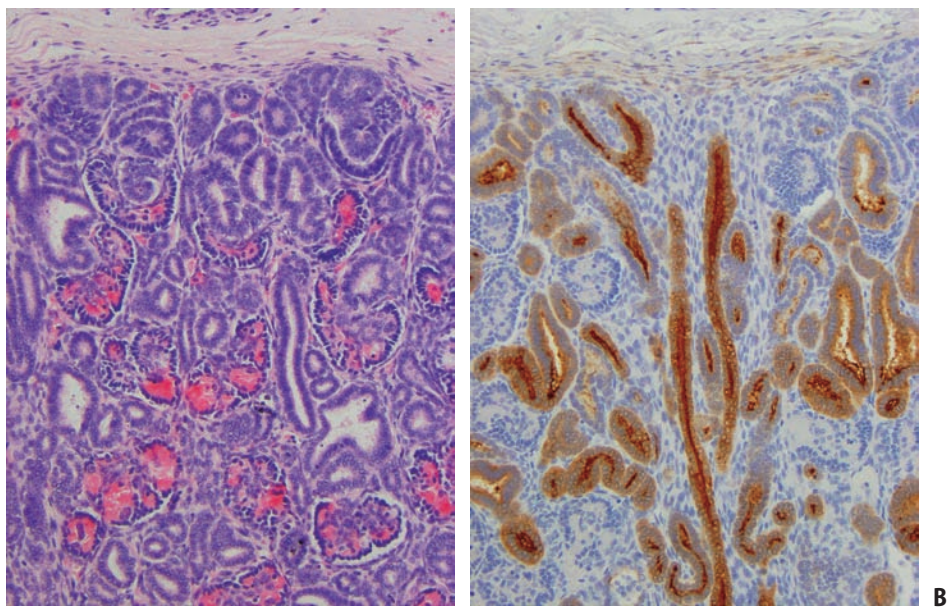

Figure 22.5. Renal tubular dysgenesis, showing crowding of glomeruli (A) with tubules staining positively to epithelial membrane antigen (B).

Renal Cystic Disease

In this section the types of renal cystic disease encountered in the perinatal period are considered. Cystic renal neoplasms and cystic renal dysplasia are considered elsewhere.

Autosomal Recessive Polycystic Kidney Disease

Autosomal recessive polycystic kidney disease (ARPKD) affects between 1:10,000 and 1:40,000 individuals. The previous term for this disease, *infantile polycystic disease*, is a less satisfactory description than ARPKD since the disease is now recognized to encompass a spectrum of renal and hepatic lesions, a minority of which may not become apparent until later childhood or even adult life. Mutations at a single locus, *PKHD1* (polycystic kidney and hepatic disease 1) located on chromosome 6p12, account for the entire spectrum of ARPKD. The predicted protein of *PKHD1*, fibrocystin/polyductin, localizes to primary cilia and the basal body, in common with other cystoproteins (Zhang et al. 2004).

Severely affected fetuses display a Potter phenotype with symmetrically grossly enlarged kidneys (Fig. 6.20 in Chapter 6). As many as 30% to 50% of all affected cases are stillborn or die in the neonatal period, often from respiratory insufficiency caused by pulmonary hypoplasia. The kidneys can sometimes be sufficiently enlarged to interfere with delivery. Despite their huge size, the reniform shape of the kidneys is preserved, and fetal lobulation is often exaggerated. On slicing the kidney, the cortex and medulla resemble a sponge and contain innumerable characteristic radially arranged fusiform cysts. On histological examination these are seen to be cystically dilated collecting ducts (Fig. 22.6). Microdissection studies in this type of renal cystic disease (Potter type 1) indicated the cysts to be due to dilation and hyperplasia of the interstitial portions of ureteric bud branches (Osathanondh and Potter 1964c). Ampullary function during organogenesis appears to be unimpaired in that branching is normal and nephron induction is unaffected.

In virtually every case of ARPKD, the renal lesion is accompanied by congenital hepatic fibrosis. The latter is characterized by a varying degree of diffuse portal fibrosis and by intrahepatic bile ducts that appear to be increased in number, are often dilated, and exhibit a characteristic angulated branching, the branches often surrounding portal veins.

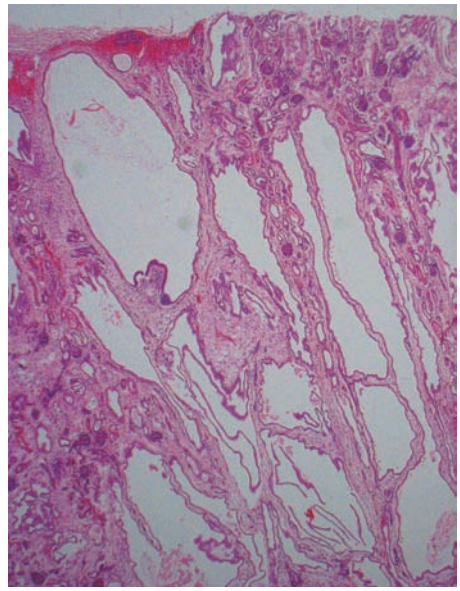

FIGURE 22.6. Autosomal recessive polycystic kidney disease, showing cystically dilated collecting ducts.

The above description encompasses cases of congenital hepatic fibrosis with classic ARPKD of the type encountered perinatally or in the preterm fetus. Although most cases presenting in the perinatal or neonatal period have a high mortality rate in the first month of life, the clinical spectrum of surviving patients is more variable (Capisonda et al. 2003). Patients carrying two truncating *PKHD1* mutations display a severe phenotype with perinatal or neonatal demise. Patients surviving the neonatal period bear at least one missense mutation. The majority of sibships exhibit comparable clinical courses, but there is gross intrafamilial phenotypic variability in about 20% (Bergmann et al. 2005). Data at this stage are incomplete, but the phenotype would appear to depend on the location and character of the mutations as well as other modifiers such as other gene and environmental factors (Bergmann et al. 2004, 2005).

To add to the confusion, congenital hepatic fibrosis accompanies other forms of familial renal

cystic disease, notably multicystic renal dysplasia in Meckel's syndrome and its variants and also some cases of juvenile nephronophthisis (Delaney et al. 1978) and Jeune's thoracic dystrophy. In practical terms, whatever the associated lesions, the presence of congenital hepatic fibrosis suggests inherited disease with an autosomal recessive transmission.

There is a strong demand for an early and reliable antenatal diagnosis in families that have previously lost a child with ARPKD. Typically, patients are identified by ultrasound late in pregnancy or at birth. Fetal ultrasound at the time when termination of pregnancy is usually performed frequently fails to detect the enlargement and the increased echogenicity of kidneys or oligohydramnios secondary to poor urine output. Gene linkage analysis can be performed to confirm the diagnosis of ARPKD if DNA is available from affected family members. Although some 193 mutations have been described, mutation analysis using denaturing high-performance liquid chromatography can identify at least one mutation in 98% of families (Bergmann et al. 2005). Where termination of pregnancy is advised on the basis of fetal ultrasound, it is essential that the fetus be properly examined. Typical changes in the fetal kidneys and liver are recognizable by 18 weeks' gestation.

Autosomal Dominant Polycystic Kidney Disease and Glomerulocystic Disease

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease, affecting 1 in 400 to 1 in 1000 livebirths. As with the older term *infantile polycystic disease* cited above, this description of ADPKD as "adult" polycystic disease is becoming outmoded following the recognition that a minority of patients with this condition may present clinically in childhood or even in infancy. Autosomal dominant polycystic kidney disease may rarely present in utero or in the early postnatal period, usually following the detection of renal cysts by detailed antenatal ultrasound scanning. About 40% of such children die before 1 year of age of pulmonary or renal insufficiency, while up to 67% develop hypertension at a mean age of 3 years (MacDermot et al. 1998). The early onset in utero form is associated with a high recurrence rate of 25% for all siblings of the affected child. The neonatal presentation is more common with maternal transmission (Brun et al. 2004).

Mutations in the polycystic kidney disease *PKD1* gene, located on chromosome 16p13.3, account for about 85% of patients, with about 15% due to mutations in the *PKD2* gene, located on chromosome 4q22. Few families are not linked to either chromosome 16 or 4, indicating at least one other unknown locus for ADPKD. *PKD1* and *PKD2* are closely related, but *PKD2* is a significantly milder disease, especially among females, than *PKD1*. Additionally, one or a few modifier genes may play a major role in the interfamilial and intrafamilial differences in the phenotype of *PKD1* (Fain et al. 2005). Mutations in *PKD1* are associated with more severe disease and earlier onset of renal failure. *PKD1* and *PKD2* code for two proteins, polycystin-1 and polycystin-2. The physiological functions of these proteins are uncertain, but they co-localize to the primary cilia of renal epithelial cells. Polycystin-1 has been implicated in the regulation of cation transport, proliferation, apoptosis, cell adhesion, and tubulogenesis. Polycystin-2 can mediate a voltage-activated calcium current as well as being involved in cell adhesion. Expression and distribution of the proteins appear to be developmentally regulated. Both proteins probably function together but each may have independent functions. The co-localization to primary cilia and the function of polycystin-1 and -2 as flow-sensitive mechanosensors in the same signal-transduction pathway, as well as studies from animal models and ciliary-disruption experiments, have led some researchers to suggest that defects in the ciliary structure and function could lead to the cystic phenotype (Ong and Harris 2005). It appears that development of the renal cysts represent a "second hit," with loss of the other functional allele in some cases, or interference with the wild-type protein (Wilson 2004).

In the context of this chapter, only those rare examples of ADPKD presenting problems in perinatal practice are considered. In these cases the kidneys vary in size from normal to being considerably enlarged (Fig. 22.7). Rounded cysts from a few millimeters up to about 3 cm in diameter may be seen in both cortex and medulla, with a

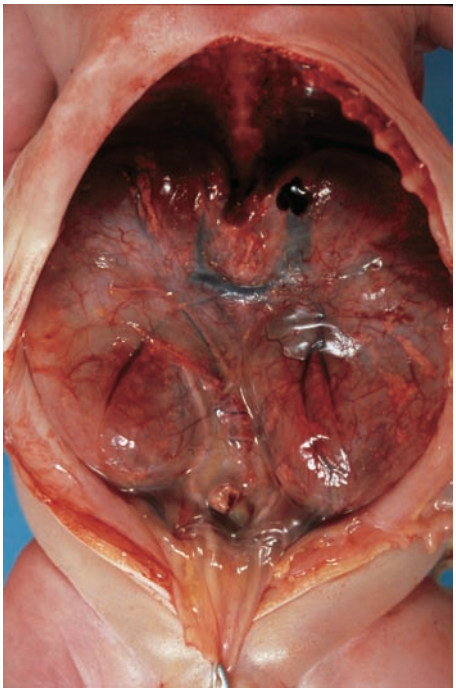

Figure 22.7. Autosomal dominant polycystic kidney disease, showing moderately enlarged kidneys.

variable distortion of the normal reniform shape (Fig. 22.8). Unlike ARPKD, in ADPKD presenting in this age group there is always a considerable portion of normal renal parenchyma, and the cysts may be derived from any part of the nephron as well as from the collecting ducts.

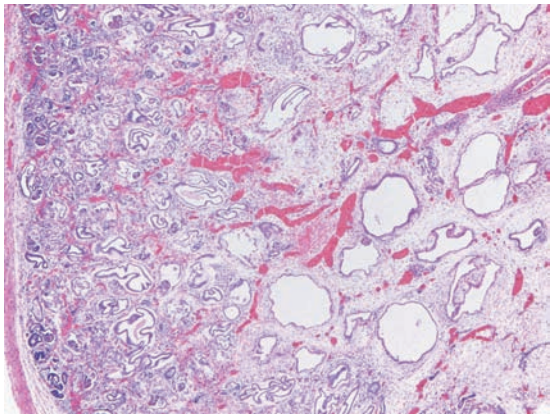

Figure 22.8. Autosomal dominant polycystic kidney disease, showing cysts in the cortex and medulla.

Often, dilatation of Bowman's spaces dominates the histological picture (Rapola and Kääriäinen 1988), so that early-onset ADPKD represents one form of so-called glomerulocystic disease. This term is now used loosely to describe a heterogeneous group of disorders that are linked only by the common feature of a variable cystic dilatation of Bowman's spaces (Taxy and Filmer 1976), rather than a specific entity (Bernstein and Landing 1989).

Glomerular cysts occur in a wide variety of inherited and sporadic disorders including early-onset ADPKD, Zellweger cerebrorenal–hepatic syndrome (Joshi and Kasznica 1984), tuberous sclerosis (Rolfes et al. 1985), trisomy 13 syndrome, Majewski-type short rib polydactyly syndrome (Joshi and Kasznica 1984), orofaciodigital syndrome (Stapleton et al. 1982), brachymesomelia–renal syndrome (Langer et al. 1983), and some examples of renal dysplasia. However, a significant proportion of cases of glomerulocystic disease occurring in infants have no abnormalities in other systems or syndromal associations and appear to be sporadic. Renal manifestations of tuberous sclerosis include angiomyolipomas, cystic disease, and renal cell carcinoma. The radiological and pathological features of renal cystic disease in tuberous sclerosis resemble those of ADPKD but the clinical onset is often early and the hyperplastic eosinophilic cells of the cystic epithelium are considered to be unique. Two tuberous sclerosis genes, *TSC1* and *TSC2*, have been mapped to chromosomes 9q34 and 16p13.3, respectively. The *TSC2* gene lies immediately adjacent to *PKD1*, and it is now clear that mutations in *PKD1* and mosaicism for large deletions of *TSC2* and *PKD1* result in renal cystic disease in tuberous sclerosis (Sampson et al. 1997). The gene products of *TSC1* and *TSC2*, hamartin and tuberin, respectively, physically interact and may be involved in the kinase signaling that regulates protein synthesis. Tuberin is also required for the correct localization of polycystin (Henske 2005).

For the pathologist, a practical approach to this rather bewildering situation is as follows. An association of glomerular cysts with renal dysplasia is usually readily apparent from the histological appearances, except perhaps in very small and unrepresentative samples. Once renal dys-

plasia is excluded, the term *glomerulocystic disease* should be applied only when this is the predominant histopathological change. Evidence of syndromal associations should then be considered, most of which will be obvious, but special caution should be exercised in excluding tuberous sclerosis, since evidence of this in family members and the index case may be subtle. About half the cases of glomerulocystic disease in infants are examples of early-onset ADPKD. In these cases the diagnosis can usually be confirmed by the recognition of other affected family members, which may require ultrasonic examination of the kidneys in asymptomatic subjects. Gene linkage analysis can be performed to confirm the diagnosis of ADPKD if DNA is available from affected family members. Mutation analysis using denaturing high-performance liquid chromatography has been used to identify mutations in *PKD1* and *PKD2* individuals with a 75% and 95% success rate (Rossetti et al. 2001).

Rare cases of localized renal cystic disease with histological features of ADPKD, but without the family history, and confined to only part of a kidney are recognized (Bisceglia and Creti 2005).

Renal Cystic Disease Associated with Multiple Malformations Syndromes

Renal cysts are a component of a number of rare, often hereditary syndromes of multiple malformations, including the autosomal trisomy syndromes, the chromosomal translocation syndromes, short rib polydactyly syndrome, Ehlers-Danlos syndrome, orofaciodigital syndrome, and type I lissencephaly syndrome (Bisceglia et al. 2006). The renal lesions are bilateral and range from minor focal microcystic changes to extensive diffuse cystic dysplasia. As stated above, in individual examples of some of these syndromes, the cysts may be predominantly glomerular.

Diffuse cystic dysplasia describes the occurrence of multiple, fairly regular, and mainly cortical cysts ranging from a few millimeters up to several centimeters in diameter in both kidneys. The medullary pyramids are poorly formed and contain scanty primitive ducts. Ductal and nephronic structures are generally scanty, and metaplastic cartilage is very unusual. While there is a superficial resemblance to multicystic dysplasia, diffuse cystic dysplasia can be distinguished by the invariably bilateral renal involvement and the presence of some degree of renal pelvic development with a patent ureter.

While diffuse cystic dysplasia is sometimes an isolated anomaly of sporadic incidence, it is often part of an hereditary syndrome of multiple malformations of which the Meckel syndrome (Fig. 22.9; also see Fig. 6.21 in Chapter 6) of microcephaly, posterior encephalocele, polydactyly, hare lip and cleft palate, and its variants is the most common (Bisceglia et al. 2006). Diffuse cystic dysplasia may also accompany the trisomy 18 and orofaciodigital syndrome. The cystic changes in the kidney are variable in Zellweger's syndrome of cerebral pachygyria and micropolygyria with ocular, genital, and hepatic malformations, and in Jeune's syndrome (Cremin 1970) of osteochondrodystrophic dwarfism and small thorax. The renal lesions range from minor focal microcystic changes to diffuse cystic renal dysplasia; involvement is bilateral. All three syndromes are inherited as autosomal recessive traits, and in all there are malformations of the intrahepatic bile ducts like those seen in infantile polycystic disease (Fig. 22.10).

Bardet-Biedl syndrome (BBS) is characterized by abnormalities of numerous organs, including the kidney where cysts and calyceal abnormalities occur frequently. Six different BBS genotypes have been identified and one, BBS8, which localizes to centrosomes and basal bodies, interacts with a protein that is involved in ciliogenesis

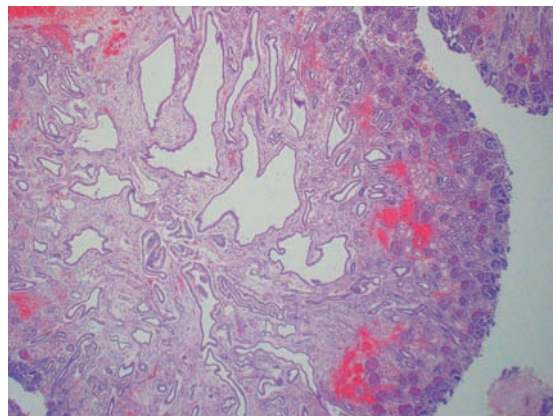

Figure 22.9. Kidney in Meckel-Gruber syndrome showing diffuse cystic changes.

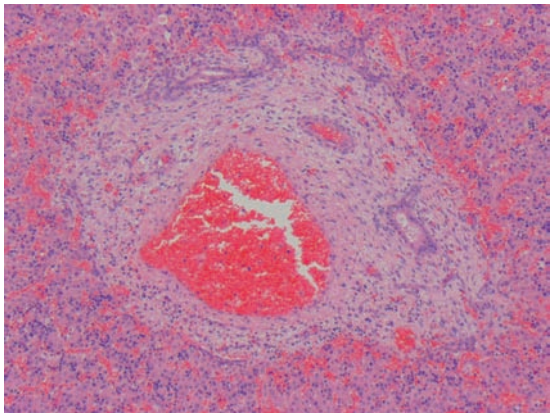

Figure 22.10. Liver showing ductal plate malformation in Meckel Gruber syndrome.

(Ansley et al. 2003). Ciliary dysfunction may also be involved in nephronophthisis. The gene responsible has been mapped to chromosome 2q13, and a nephronophthisis gene, *NPHP3*, encodes a protein that interacts with the ciliary protein nephrocystin (Olbrich et al. 2003). Tuberous sclerosis has been mentioned in the context of glomerulocystic disease (see p. 635).

Hereditary Abnormalities of Renal Tubular Transport

Although a number of hereditary disorders of renal tubular transport are recognized, the pathological changes seen in the kidney are seldom of help in diagnosis. A classification of these disorders is presented in Table 22.1.

Renal Hypoperfusion

Hypoperfusion of the kidneys in the perinatal period may result in renal tubular necrosis, renal cortical necrosis, renal medullary necrosis, or renal vein thrombosis; combinations of these lesions frequently occur. Asphyxia and hypotension from any cause in the fetus and newborn may result in spasm of the splanchnic circulation, which appears to underlie the susceptibility to ischemic lesions in the kidney as well as the intestine and adrenal in this age group. Intrapartum asphyxia, placenta previa, severe hemolytic disease, and some congenital heart lesions are potent causes in the perinatal period (Fig. 22.11). Renal tubular dysgenesis (see above) is thought to be due to prolonged renal hypoperfusion in the fetus.

In infancy, and particularly during the first 2 months of life, renal hypoperfusion is often complicated by renal vein thrombosis, and dehydration resulting from gastroenteritis is a common predisposing cause. Clinically, the condition is recognized by the presence of firm enlargement of the kidney in a sick, often dehydrated infant with oliguria and hematuria. Evidence of disseminated intravascular coagulation and thrombocytopenia may also be demonstrated. In the fetus, renal vein thrombosis is associated with maternal diabetes and thrombophilia.

Congenital Nephrotic Syndrome

The most common causes of congenital nephrotic syndrome are congenital nephrotic syndrome of the Finnish type and diffuse mesangial sclerosis or the French type. Finnish-type congenital nephrotic syndrome is the single most common cause of congenital nephrotic syndrome and is particularly common but not restricted to families of Finnish origin. It is inherited as an autosomal recessive trait, and the abnormal gene, *NPHS1*, has been localized to chromosome 19q13.1. *NPHS1* encodes for nephrin, which is a transmembrane protein produced by podocytes, and is localized to the slit membrane. Two common mutations, Fin major and Fin minor, account for over 95% of cases in Finland. In diffuse mesangial sclerosis, mutations in the *NPHS2* gene, localized to chromosome 1q25-q31, have been identified. *NPHS2* encoded podocin that also localizes to the slit membrane. Genotype/phenotype correlation in *NPHS1* and *NPHS2* mutations is poor (Schultheiss et al. 2004).

The nephrotic syndrome is recognized at or very soon after birth. Microscopic hematuria is often present. The infant is generally premature and of low birthweight and the placenta is large and edematous, frequently constituting 25% to 60% of the birth weight (Kouvalainen et al. 1962). Maternal and amniotic α-fetoprotein levels are elevated, reflecting the fetal proteinuria, and can be used for antenatal diagnosis where there is a

Table 22.1. Familial abnormalities of tubular transport (Kissane 1973; Risdon 1981)

Disorder	Inheritance	Functional abnormality	Morphological changes in kidneys	Clinical effects
1. Specific disorders of amino acid transport				
a. Cystinuria	Autosomal recessive	Proximal tubular defect → increased urinary excretion of cystine, lysine, arginine and ornithine		Cystine calculi in the urinary tract
b. Hartnup disease	Autosomal recessive	Proximal tubular defect → increased urinary excretion of alanine, glutamine, asparagine, histidine, serine, threonine, phenylalanine, tyrosine and tryptophan		Pellagra-like skin rash Attacks of cerebellar ataxia
c. Iminoglycinuria	Autosomal recessive	Proximal tubular defect → increased urinary excretion of glycine, proline and hydroxyproline		Urinary tract calculi
2. Nonspecific disorders of amino acid transport				
a. Cystinosis (de Toni—Faconi—Lignac—Debré syndrome)				
i. Childhood form	Autosomal recessive	Proximal tubular defect → aminoaciduria, hyperphosphat uria, acidosis and hypokalemia May be proteinuria	Deposition of cystine crystals in tubules, glomerular epithelial cells, and interstitium (as well as elsewhere in the body) Swan-neck deformity of nephrons on microdissection	Vomiting, fever, polyuria Vitamin D–resistant rickets Occasionally pitressin-resistant diabetes insipidus Renal failure
ii. Idiopathic form	Autosomal recessive and dominant	Proximal tubular defect → glycosuria, aminoaciduria, hypophosphaturia	No cystine deposition	Milder disease than (i) Usually affects adults but may be present in childhood
b. Lowe's syndrome	Sex-linked recessive	Proximal tubular defect → aminoaciduria, hypophosphaturia Acidosis, proteinuria and inability to concentrate the urine	Tubular atrophy and glomerulo sclerosis	Congenital glaucoma, cataracts, mental retardation, rickets, and renal failure

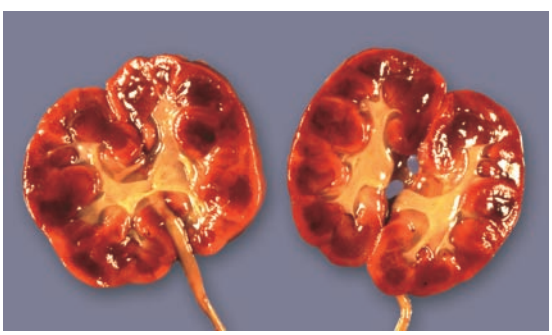

Figure 22.11. Renal cortical and medullary necrosis.

known family history. The proteinuria fails to respond to corticosteroid or immunosuppressive therapy. Without renal transplantation, survival beyond the first year is uncommon. Histologically, the renal glomeruli appear immature and may exhibit mild to moderate mesangial hypercellularity early in the disease. Later, an increasing proportion of glomeruli show segmental or global sclerosis. Characteristically many tubules are cystically dilated ("microcystic" disease). Immunofluorescence microscopy reveals no consistent glomerular deposition of immunoreactants and is

Table 22.1. *Continued*

Disorder	Inheritance	Functional abnormality	Morphological changes in kidneys	Clinical effects
3. Tubular defects owing to endogenous poisons				
a. Galactosemia	Autosomal recessive	Galactose-l-phosphateuridyl transferase deficiency → galactose retention Effect on proximal tubules → aminoaciduria and proteinuria		Cataracts, mental deficiency, and hepatic cirrhosis
b. Wilson's disease	Autosomal recessive	Defect of copper metabolism associated with reduced serum ceruloplasmin and deposition of copper. Effects on proximal tubules → aminoaciduria		Extrapyramidal symptoms, Kayser—Fleischer rings, hepatic cirrhosis
4. Disorders of other transport mechanisms				
a. Renal glycosuria	Autosomal dominant	Prominent tubular defect → glycosuria		
b. Vitamin D—resistant rickets	Sex-linked dominant	Increased clearance of phosphate owing to reduced reabsorption in the proximal tubules		Rickets refractory to therapy with vitamin D
c. Vasopressin-resistant diabetes	Probably sex-linked dominant with variable expressivity	Distal tubular defect resulting in unresponsiveness to vasopressin	Microdissection indicates diminution in proximal tubule convolutions	Vasopressin-resistant diabetes insipidus
d. Primary renal acidosis				
i. Infantile form	Autosomal recessive	Distal tubular defect → inability to acidify urine	Reduction in renal size and nephrocalcinosis in some cases	Vomiting, failure to thrive, dehydration and hypotonia Complete recovery following treatment is usual. Possible failure in maturation of tubular function
ii. Late form	Autosomal dominant penetr ation in females	As in (i). Increased potassium loss in urine may occur	May be cortical scarring and with increased urolithiasis	More serious disorder than (i) May develop periodic paralysis, rickets, and renal stones

generally completely negative. Immunostaining for nephrin is negative, although positive staining has been reported in some non-Finnish patients with compound heterozygous mutations. Electron microscopy fails to reveal evidence of immune complex deposition, but there is diffuse effacement of podocyte foot processes. This change is seen even in utero, indicating that proteinuria occurs at this stage.

Diffuse mesangial sclerosis, also referred to as French-type congenital nephrotic syndrome, and so-called because it was first described in the French literature (Habib and Bois 1973), also presents in the first year of life, sometimes in the first weeks. The clinical course is characterized by progressive renal failure and death between the ages of 1 and 3 years.

Histologically, the fully developed glomerular lesion is characterized by diffuse mesangial sclerosis with a marked increase in mesangial matrix and obliteration of most tuft capillary lumina. The glomerular tuft is contracted to a rounded mass, often covered by hypertrophied and sometimes vacuolated podocytes. Tuft nuclei are surprisingly

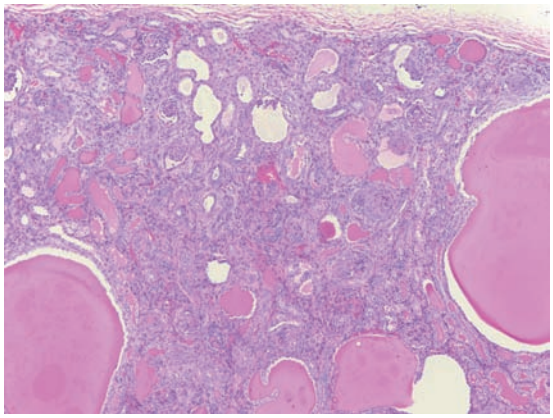

Figure 22.12. Diffuse mesangial sclerosis, showing mesangial matrix proliferation and obliteration of tuft capillary lumina.

well preserved for the degree of sclerosis, and because tufts are contracted they often appear superficially to be hypercellular (Fig. 22.12). Capsular adhesions and crescents are uncommon, and silver staining may demonstrate focal double contouring of residual thickened peripheral tuft capillary walls. Examination of sections of the whole kidney show the most severely affected glomeruli to be in the middle to outer cortex; in the juxtamedullary cortex, glomeruli may show only an increase in fibrillary mesangial matrix, and in early lesions may appear normal. In the extreme outer, subcapsular cortex, small immature and partly sclerosed glomeruli with a simplified lobular structure associated with small atrophic tubules often form a distinct layer.

Tubulointerstitial changes are generally marked, with tubular atrophy as well as dilated and often hypertrophied tubules containing eosinophilic proteinaceous casts associated with diffuse interstitial fibrosis. Immunofluorescence microscopy may reveal diffuse deposition of immunoglobulin M (IgM) and C_3 in the glomerular mesangium and along thickened tuft capillary walls. Immunostaining for podocin is markedly diminished.

In diffuse mesangial sclerosis, the increase in mesangial matrix is confirmed ultrastructurally, and occasional mesangial interposition is seen corresponding to the double contours seen by light microscopy. Abnormalities of the glomerular basement membrane may be apparent focally. The membrane outside the normal lamina densa is thickened, with a wavy outer contour. Strands of lamina densa–like material delineate clear lacunae in the outer glomerular basement membrane to give an appearance superficially resembling that in Alport's syndrome. An intriguing feature of diffuse mesangial sclerosis is that exactly the same glomerular lesion is seen in Denys-Drash syndrome (male pseudohermaphroditism, nephropathic syndrome, and nephroblastoma). In the Drash syndrome, nephrotic syndrome and renal failure often develop early, and incomplete forms without Wilms' tumor or occurring in genotypic females are described. This raises the possibility that the Drash syndrome in its various forms and congenital nephrosis with diffuse mesangial sclerosis are part of a continuum. Mutations of the *WT1* gene are a constant feature of the Drash syndrome (Pelletier et al. 1991) and have been identified in diffuse mesangial sclerosis. Diffuse mesangial sclerosis changes are seen also in Galloway-Mowat syndrome (nephrotic syndrome and central nervous system abnormalities).

Other congenital mutations in genes related to congenital nephrotic syndromes include Allport's (collagen IV) and the nail patella syndrome *(LMX1B)*. These genes often share a pathway or interact with one another in maintaining normal podocyte function (Bouchard 2004).

Renal Infection

Bacterial infection of the kidney is rarely demonstrated in perinatal necropsies. However, obstructive congenital anomalies of the urinary tract and vesicoureteric reflux are important predisposing factors in the development of the pyelonephritic scarring, which may be seen in older children (Risdon 1981a). It is important to recognize that in the presence of vesicoureteric reflux urinary infection may induce renal scarring very early in life, although this may not be recognized clinically until much later. In the perinatal period acute bacterial infection of the kidney sometimes complicates a generalized septicemia and may also be seen with lower urinary tract obstruction, particularly with posterior urethral valves in infant boys, in whom the kidney often exhibits dysplastic changes in addition to those associated with infection (Risdon 1971b).

Viral and fungal infections of the kidney may also be recognized in the perinatal period. Hemorrhagic infarction of the renal medulla and inner adrenal cortex has been described in infants dying during an outbreak of echovirus 11 infection. In congenital cytomegalovirus infections, the typical nuclear inclusions can often be recognized in some proximal tubular cells in the kidneys.

Intravenous feeding in neonates may be complicated by fungal infections, particularly with *Candida* species, and the organisms can often be recognized in sections of the kidney.

Renal Glomerular Lesions in Cyanotic Congenital Heart Disease

Glomerular enlargement, often associated with mesangial cell proliferation and occasionally with mesangial accumulation of PAS-positive fibrillary material, may be seen in infants dying of cyanotic congenital heart disease. Clinical evidence of renal impairment or proteinuria is lacking, and there is no increase in renal size. Hypoxia, polycythemia, and pulmonary hypertension are all factors that have been associated with the glomerular change. Similar changes may be encountered in patients with primary pulmonary hypertension and also in the twin–to-twin transfusion syndrome in the kidneys of the recipient twin, when there is significant polycythemia.

Congenital Abnormalities of the Renal Pelvis and Ureter

Duplication

Duplication of the renal pelvis and ureter ranges from mere bifurcation of the extrarenal pelvis, to duplication of the pelvis and upper ureter joining to form a single ureter entering the trigone, to complete duplication of the whole system with two separate ureteric orifices. Minor duplications are common, but complete duplications are much rarer, and very occasionally triplication or even quadruplication of the ureter is recognized. All these anomalies are due to premature division or duplication of the ureteric bud. Rarely, in partial ureteric duplication, one of the ureters ends blindly and does not communicate with the renal pelvis and kidney. This condition is also referred to as a diverticulum of the ureter.

Vesicoureteric Reflux

Vesicoureteric reflux (VUR) is the abnormal flow of urine from the bladder into the upper urinary tract. It may be an isolated anomaly or be associated with other congenital anomalies such as posterior urethral valves or duplication of the urinary tract. Reflux predisposes to pyelonephritis. Reflux is the most common cause of congenital hydronephrosis, being responsible for about 40% of intrauterine cases. The clinical significance of antenatally detected asymptomatic renal pelvis dilatation, described as occurring in between 1% and 20% of fetuses, is unclear, however (Jaswon et al. 1999; Cohen-Overbeek et al. 2005).

Ureteric Ectopia

An ectopic ureter is inserted distally at a site other than the normal in the trigone of the bladder. In females, the ectopic ureter may drain into the lower bladder, urethra, vestibule, or vagina. An ectopic ureter draining to the vagina or vestibule appears more common in Asian countries (Nakai et al. 2003). In males, it empties into the lower bladder, posterior urethra, seminal vesicle, vas deferens, or ejaculatory duct. Rarely, it can empty into the rectum. Minor degrees of lateral ectopia in the bladder may be associated with primary vesicoureteric reflux. An ectopic ureter can drain a single kidney, but about 70% are associated with complete duplication of the ureter with two ureteric orifices. The orifice draining the upper part of the kidney is usually medial and inferior to its normal location and to the orifice of the ureter draining the lower pole. The ureter draining the upper pole frequently ends in a ureterocele while reflux into the lower pole typically occurs (Berrocal et al. 2002). The part of the kidney drained by the ectopic ureter may be hypoplastic or dysplastic, particularly if vesicoureteric reflux occurs.

Ureterocele

A ureterocele is a saccular expansion of the distal ureter involving the intramural segment in the

bladder wall and the submucosal segment immediately above the orifice. Ureteroceles are classified according to the number of ureters that drain the kidney ipsilateral to the ureterocele, the location of the ureterocele, and additional anatomical distortions due to the ureterocele. A ureterocele that is associated with a completely duplicated collecting system, usually of the upper pole, is a duplex-system ureterocele, while one that drains a kidney that is subtended by a single ureter is a single-system ureterocele. An intravesical ureterocele is entirely located within the bladder while an ectopic ureterocele typically courses through the submucosa of the bladder to terminate at the bladder neck or urethra. While the majority of ectopic ureteroceles are associated with ectopic ureters and ureteral duplications, as described in the previous paragraph, some can be normally located and both intravesical and ectopic single-system ureteroceles occur (Zerin et al. 2000). An ectopic ureterocele is more common in girls than in boys and is usually unilateral. Most ureteroceles are now detected antenatally. A paraureteral diverticulum is associated with vesicoureteric reflux (Afshar et al. 2005).

Retrocaval Ureter

In the rare anomaly of retrocaval ureter the upper part of the ureter passes behind the inferior vena cava and descends medially to it to the bladder.

Congenital Hydrocalycosis

Congenital obstruction of the infundibular portion of a renal calyx produces dilatation of the calyx and atrophy of the associated renal parenchyma (hydrocalycosis). One or several calyces may be affected and obstruction may be extrinsic, due to, for example, aberrant blood vessels, or intrinsic, due to congenital infundibular stenosis.

Congenital Hydronephrosis

See p. 631.

Ureteral Dilatation

Congenital ureteric dilatation or megaureter is common and may be primary or secondary. Primary megaureter includes all cases of megaureter due to an idiopathic congenital alteration at the vesicoureteric junction. Primary megaureters may be obstructed or nonobstructed, refluxing or nonrefluxing, or nonrefluxing unobstructed. Secondary megaureter occurs as a result of some abnormality involving the bladder or urethra, for example, urethral valves, ureteroceles, etc. It is important to recognize that the diameter of the ureter in the neonatal period is relatively greater than in older children and adults, a point to be remembered in assessing the normality or abnormality of the caliber of the ureter in this age group. The management of mild ureteric dilatation is a clinical problem associated with vesicoureteric reflux and other disorders (Cohen-Overbeek et al. 2005).

Congenital Abnormalities of the Bladder

Major congenital anomalies of the bladder are rare, though minor anomalies are more common. Major structural anomalies include agenesis, duplication, and septation. A prenatal detection of an abnormally large bladder should suggest a bladder outlet obstruction (usually in males), but the megacystis-microcolon syndrome should also be considered (see below) (Verbruggen et al. 2004).

Congenital absence of the bladder is an extremely rare anomaly. Affected infants are usually stillborn, and other congenital anomalies usually coexist. Ectopic insertions of the ureters into the vagina in females and into the rectum in males have been described.

Bladder duplication may be complete or incomplete (Bae et al. 2005). In complete duplication two separate bladders with separate urethras lie side by side in a common adventitial sheath. Duplication of the hindgut is almost always present and a rectourethral fistula involving one bladder is present in over half the cases. Other rectal and genital anomalies commonly coexist. In partial duplication two bladders are joined at their base and have a single urethra.

Various types of septation of the bladder have been described. Sagittal septation may be com-

plete or incomplete. When complete, one half of the bladder is separated from the urethra and hydronephrosis occurs on that side. Frontal septation divides the bladder into anterior and posterior compartments and is usually incomplete. The so-called hourglass bladder probably reflects a partial persistence of the urachus and may be considered as an exaggerated form of urachal diverticulum. Diverticula have also been described (Stein et al. 2005).

There are congenital presumed neurological conditions affecting bladder function, some of which are associated with abnormal facial movements, but these conditions are diagnosed on the clinical features (Ochoa 2004).

Persistence of the urachus results in a fistula from the bladder to the umbilicus. Partial persistence of the distal urachus forms a urachal diverticulum, and persistence of the middle part of the urachus produces a urachal cyst.

Bladder Exstrophy

Bladder exstrophy results from a failure in fusion of the mesodermal elements of the anterior abdominal wall below the umbilicus. These congenital anomalies range from minor degrees of epispadias with exposure of the terminal penile urethra to gross ectopia vesicae with accompanying hindgut anomalies and often a vesicointestinal fistula. These may show familial clustering as the bladder exstrophy and epispadias complex (BEEC) (Reutter et al. 2003). The incidence is around 2 per 100,000 births, and though a sex imbalance has been reported, it is approximately equal in large epidemiology studies (Nelson et al. 2005). It may be more common in whites in the United States. Bladder exstrophy and cloacal exstrophy appear to be related conditions due to the same underlying developmental field defect (Martinez-Frias et al. 2001) with cloacal exstrophy around 10 times less common. There are associated anomalies including cleft palate, spina bifida, and preterm birth. Occasional autosomal dominant inheritance is reported (Froster et al. 2004). The OEIS syndrome (omphalocele, exstrophy, imperforate anus, and spinal defects) may represent the severe end of this spectrum (Kallen et al. 2000; Keppler-Noreuil 2001). The body stalk/short umbilical cord anomaly and some conjoined twins with omphalopagus may also have a similar range of anomalies, suggesting a sequence effect from an early defect involving the posterior part of the embryonic disk.

In bladder exstrophy the abdominal wall below the umbilicus is shortened and there is a variably sized midline defect. This ranges from a small hole, through which the bladder trigone protrudes on straining, to a large defect through which the entire posterior wall of the bladder is exposed (ectopia vesicae). Some degree of pubic diastasis and epispadias, in which the urethra is open on the upper surface of the penis, invariably accompanies bladder exstrophy (Kiddoo et al. 2004).

In surviving children, secondary changes occur in the exposed bladder mucosa, such as squamous metaplasia, cystitis cystica, and cystitis glandularis. Squamous or adenocarcinoma may develop in patients surviving childhood. In cases amenable to surgical correction, vesicoureteric reflux is usually demonstrable after surgery.

In cloacal exstrophy, an exstrophied bladder is in two separate halves, each with a ureteric orifice; these hemibladders are separated by exstrophied bowel. This bowel has two openings—an upper orifice communicating with terminal ileum, which frequently prolapses as a tube covered by intestinal mucosa, and a lower orifice communicating with a blind-ending segment of colon. The anus is imperforate and there may be a separate appendicular orifice (or sometimes two, as the appendix is frequently duplicated). A large exomphalos above the exstrophy usually contains liver and intestine. An epispadiac penis is present in males and is frequently duplicated. The testes are undescended and the scrotum is absent. In females the vagina is septate, the uterus duplicated, and the external genitalia are absent. There is a long-term risk of squamous cell carcinoma of the bladder in survivors.

Covered exstrophy occurs with delayed closure of the abdominal wall defect following the formation of an exstrophy. The umbilicus is low and the abdomen below it consists of a paper-thin membrane formed from skin and anterior bladder wall. There is an increased risk of cloacal exstrophy in conjoined twins (Casale et al. 2004) and also possibly in vitro fertilization (Wood et al. 2003).

Congenital Abnormalities of the Urethra

Urethral Valves and Strictures

Posterior urethral valves are the commonest cause of congenital urethral obstruction, occurring only in boys. This is an accentuation of a pair of mucosal folds normally present in the male urethra, extending down laterally from the verumontanum to the urethral wall. The exact embryology is still the subject of discussion (Krishnan et al. 2006). In effect, this is a diaphragm that becomes sail-like as it is progressively distended during voiding (Cremin 1970; Berrocal et al. 2002). Wigglesworth (1984) suggests that this appearance might be transformed to the more classically described one by the passage of a catheter or by opening the urethra anteriorly at necropsy.

Prenatal ultrasound diagnosis is the usual method of detecting posterior urethral valves. The consequences of posterior urethral valves depend largely on the degree of obstruction caused and may include oligohydramnios, bladder distention, and, occasionally, fetal ascites. Vesicoureteric reflux with hydronephrosis and renal dysplasia are common.

Anterior urethral valves are estimated to be seven times less common than posterior urethral valves. The majority is a result of urethral diverticula, but cases composed of simple transverse membranes have been described. Associated urinary tract anomalies are generally less severe than with posterior urethral valves, but profound bladder wall thickening, hydronephrosis, and renal failure can result (Jones et al. 2002).

Congenital urethral stenosis may result from strictures developing because of inadequate cooption of the genital tubercles (in males) or from mucosal diaphragms. The strictures may be single or multiple, and meatal strictures are the most common. Findings above the stenotic segment are similar to those of posterior urethral valves and include bladder wall thickening, hydronephrosis, and renal dysplasia.

Prune Belly Syndrome

The combination of atrophy of the muscles of the anterior abdominal wall, undescended testes with bilateral hydronephrosis, and hydroureter in an infant male is termed the "prune belly" syndrome (Wigger and Blanc 1977) because of the prune-like wrinkling of the anterior abdominal skin consequent on the absent musculature.

Mechanical urethral obstruction is unusual, although occasionally there is atresia of the membranous urethra or urethral valves. More often there is tapering dilatation of the posterior urethra narrowing into the membranous urethra without obstruction, although clinical investigation indicates an abnormal functional resistance in the membranous urethra. The bladder is thick-walled, although usually not trabeculated. The trigone is abnormally wide and the ureteric orifices dilated and subject to vesicoureteric reflux. The ureters are dilated and tortuous; the kidneys are hydronephrotic and show varying degrees of dysplastic development. Rare familial cases are described.

Megacystis/Megaureter Syndrome

Megacystis/megaureter syndrome is characterized by a very large capacity, thin-walled bladder associated with gross bilateral vesicoureteric reflux, hydroureters, and hydronephrosis. The kidneys are also often dysplastic. The bladder often appears persistently full, but this is because detrusor contraction at micturition tends to cause urine to flow retrogradely into the capacious ureters as well as through the urethra. When the bladder is emptied, it immediately refills when urine returns from the ureters by gravity. Megacystis may be seen in association with microcolon, and there is often a functional obstruction of both the gastrointestinal and genitourinary tracts.

Urethral Atresia

Urethral atresia is an uncommon condition characterized by a congenital absence of the urethral lumen, generally at the level of the membranous urethra. It may be accompanied by a rectourethral or urachal fistula and is sometimes seen in the prune belly syndrome.

Urethral Duplication

Duplication of the urethra (accessory urethra) is rare. In most cases, the duplication occurs in a

coronal plane, with the ventral urethra being of the most normal caliber. Urethral duplication is exceptional in females and is usually associated with other caudal anomalies or bladder duplication. The duplication may join to open at one meatus or be associated with two, when one meatus would be normally placed while the second meatus is in the perineum. The child often presents with wetting or with infection.

Prostatic Utricle (Müllerian Duct) Cyst

The prostatic utricle is a short blind-ending pouch located on the verumontanum that is a remnant of the müllerian ducts. Utricular cysts are therefore located on the floor of the prostatic urethra in the midline. Regression of the utricle is androgen-mediated, and utricular cysts are therefore found with increased frequency in boys with other disorders such as hypospadias and prune belly syndrome (Berrocal et al. 2002).

Polyp of the Verumontanum

This is a connective tissue polyp covered by urothelium arising from the floor of the prostatic urethra near the verumontanum.

Megalourethra and Urethral Diverticulum

Megalourethra is a rare congenital disorder that occurs in males. The urethra dilates in either a scaphoid (or saccular) manner because of poor development of the corpus spongiosum or in a fusiform (or globular) manner because of deficient development of the corpus spongiosum as well as the corpora cavernosa. The scaphoid and fusiform are variants along a continuum as deficiencies of the spongiosal tissue may be variable or only one of the two corpora cavernosa may be affected (Jones et al. 2002). The development of the megalourethra appears to be rare and sporadic with no hereditary disposition. They are usually apparent in the neonatal period as an obviously enlarged or deformed phallus. Prenatal ultrasound diagnosis has been reported. Associated anomalies are usually within the prune belly syndrome.

The urethral diverticulum refers to an epithelialized saccular dilatation that is separate from the urethra but communicates by means of a discrete orifice. Congenital urethral diverticulum occurs almost exclusively in the anterior urethra. Although uncommon, it is the second most common cause of congenital urethral obstruction in boys. Patients generally present with infection, bulging, or deviation of the penis.

Hypospadias

Hypospadias is one of the most frequent congenital anomalies among newborn males. The birth prevalence, between 0.3% to 0.8% of male births, is not uniform worldwide, and the frequency has reportedly been increasing, steady, or decreasing (Pierik et al. 2002; Ahmed et al. 2004; Martinez-Frías et al. 2004). It is defined by a ventral displacement of the urethral meatus from the tip of the glans penis to the underside of the phallus, scrotum, or perineum. The ventral foreskin is deficient and the penis is often short and curved downward because of tethering bands of connective tissue (ventral chordee). The condition is classified according to the position of urethral meatus. The first degree is the mildest, and the urethra opens on the anterior portion of the glans (glandular and subcoronal). The second degree involves openings on the midshaft of the penis and can be distal penile, midshaft, or proximal penile. The third degree is the most severe form and involves penoscrotal, scrotal, and perineal openings. Undescended testes (cryptorchidism) and inguinal hernias are urogenital anomalies that are most commonly associated with hypospadias. Extragenitourinary anomalies, involving craniofacial, cardiothoracic, and gastrointestinal systems, are described in about 6.7% of all patients with hypospadias (Manson and Carr 2003). Hypospadias is thought to have a multifactorial etiology with genes controlling androgenic action and metabolism likely to play important roles.

Acquired Diseases of Immature Kidneys

Premature infants are prone to diseases such as acute tubular damage, due to poor renal perfusion, which may be compounded by aminoglycosides (gentamicin). Nephrocalcinosis is also common and is associated with loop diuretics,

dexamethasone, and impaired acid base balance (Alpert and Noe 2004; Hein et al. 2004).

With increasing interest of the medical community in the fetal origins of adult disease, the kidney probably has an important role in the pathophysiological pathway of adult-onset hypertension and cardiovascular disease. Growth restriction (and also probably prematurity) are associated with small kidneys with reduced numbers of nephrons in humans and animals (Amann et al. 2004; Latini et al. 2004).

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23
The Reproductive System

Ewa Rajpert-De Meyts and Niels Graem

Development of the reproductive system, which comprises the gonads and external genitals in male and female embryos and fetuses, is under very complex and exquisitely synchronized genetic and hormonal control. The reproductive system is unique among the functional organ systems in the body in that it has the most pronounced sexual dimorphism. The gonads can develop either into a testis or an ovary, which differ markedly from each other in several aspects of the biology of gamete production. On the other hand, the accessory reproductive organs in the female are mainly geared for future implantations and gestations, while the male function is limited to the delivery of spermatozoa. During the last decades, a number of genes responsible for primary sex differentiation have been discovered, as well as endocrine and paracrine signaling pathways regulating these events and the subsequent development of gonads, ducts, and external genitalia. Many of the breakthroughs in our understanding of this system were made in observing abnormal development of the reproductive system in humans or in animal models with targeted gene disruption. Despite these advances, a great deal remains to be elucidated. While certain gene pathways leading to reproductive abnormalities have been relatively well described, little is known about the role of environmental and lifestyle factors, especially in mild cases. A possible relationship between prenatal exposures to environmental endocrine disrupters and abnormalities of the reproductive system, including genital malformations, testicular germ cell tumors, and some forms of infertility in adult life has been proposed (Toppari et al. 1996; Skakkebaek et al. 2001).

Normal Development

Indifferent Gonads

The indifferent (bipotential) gonads develop within the adrenogenital primordium in two paired urogenital ridges of mesenchyme (derived from the intermediate mesoderm) from the mesonephros during the 4th to 6th weeks of gestation. The formation of the indifferent gonad is triggered by action of a number of genes, two of which are absolutely essential for the development of the entire urogenital system, including also kidneys and adrenals: the Wilms' tumor 1 gene (*WT1*) and the steroidogenic factor 1 (SF-1).

Initially, the indifferent gonads do not contain germ cells. The primordial germ cells (PGCs) originate from pluripotent cells of the epiblast near the allantoic invagination of the yolk sac. Germ cell specification has not yet been completely unraveled, but several genes have been implicated as possibly involved in this process in the mouse. Among the first proposed were several members of the family of bone morphogenetic proteins, BMP-2, BMP-4, and BMP-8b (Lawson et al. 1999; Ying and Zhao 2001; Ying et al. 2001). More recently, *fragilis, stella (Dppa3)*, and transcriptional repressor Blimp1 were indicated as determinants of the germ cell specification (Saitou et al. 2002; Ohinata et al. 2005). Proliferating PGCs migrate along the midline axis to the gonadal

ridges. This is due to the combined effect of cell adhesion factors (integrins and cadherins), and mitotic and antiapoptotic factors, mainly the KIT receptor/stem cell factor (SCF) signaling pathway (Molyneaux and Wylie 2004). The PGCs do not differentiate due to the continuous high expression of several embryonic pluripotency factors, such as OCT4 and NANOG (Chambers et al. 2003). Primordial germ cell migration is completed around 6 weeks' gestation. At 6 to 7 weeks' gestation, all embryos, regardless of the karyotype, have indifferent gonads consisting of germ cells, coelomic surface epithelium, and mesenchyme (Fig. 23.1A). Also at this stage, the internal müllerian (paramesonephric) and wolffian (mesonephric) ducts, the urogenital sinus, and the undifferentiated external genitalia can be identified (Fig. 23.2).

The subsequent sex specific development of internal and external genitalia is primarily dependent on the karyotype and the genotype, but is also influenced by the endocrine and paracrine function of the gonads.

Testes

After completion of the migration at 6 to 7 weeks' gestation, PGCs are enclosed by immature Sertoli cells originating from invading celomic surface, and by peritubular cells, thus forming seminiferous cords (Fig. 23.1B). At 7 weeks' gestation, the Sertoli cells begin to secrete a peptide hormone, the anti-müllerian hormone (AMH), also called müllerian inhibiting substance (Rajpert-De Meyts et al. 1999). This hormone induces regression of the müllerian ducts and later probably also contributes to the testicular descent. Germ cells multiply by mitosis during fetal life and most actively during the first and second trimesters. From around 20 weeks of gestation, fetal gonocytes, which until then resemble closely PGCs, gradually begin to differentiate into infantile spermatogonia, and they downregulate the embryonic pluripotency genes while acquiring germ-cell–specific genes, many of these mapped to both sex chromosomes (Rajpert-De Meyts et al. 2004). This process is completed in early infancy. At 7 to 8 weeks' gestation, numerous Leydig cells originating from mesenchymal cells can be seen in the interstitium.

It has long been known that the presence of a Y chromosome is decisive for testicular differentiation. The sex-determining region of the Y *(SRY)* encodes a transcription factor, which is involved in inducing differentiation of the Sertoli cell lineage from mesonephric cells (Capel et al. 1999). Subsequently, other genes involved in primary sex determination have been discovered, mainly by studying patients with errors of sex differentiation. Among the best characterized are *SOX9*, which displays similarities with *SRY*, and *DMRT1* and *DMRT2*, two genes mapped to a region on chromosome 9p that when deleted causes male-to-female sex reversal (Raymond et al. 2000).

The Leydig cells secrete several hormones, including testosterone, under the influence of human chorionic gonadotropin from the placenta. After 10 to 12 weeks' gestation the hypothalamo–pituitary–gonadal axis is operating, and from that time testosterone biosynthesis is mainly under control of fetal luteinizing hormone (LH). At this time of fetal development, the level of testosterone in serum reaches its maximum and is as high as in the adult male. Testosterone is crucial for male differentiation of wolffian ducts, urogenital sinus, testicular descent, and external genitalia (Figs. 23.2 and 23.3). Other hormones important for testicular development and initiation of testicular hormone production are the SF-1 and DAX1 (Swain et al. 1996), as well as insulin-like Leydig factor 3 (INSL3, also known as relaxin-like factor, RLF) (Nef and Parada 1999; Zimmermann et al. 1999), which is crucial for testicular descent.

Ovaries

The PGCs aggregate in the peripheral zone, which becomes the cortex. Thereupon they enlarge and become oogonia. After rapid mitotic proliferation, the oogonia enter the first meiotic prophase during 9 to 12 weeks' gestation to become primary oocytes (Bendsen et al. 2006). Under the light microscope, these cells may be recognized by their prominent nuclei with condensed fibrillar chromatin (Fig. 23.1C). By mid-pregnancy the fetal ovaries contain 6 to 7 million closely packed germ cells, but hereafter and for the rest of female life the number is constantly decreasing. After 20 weeks' gestation, primitive granulosa cells derived

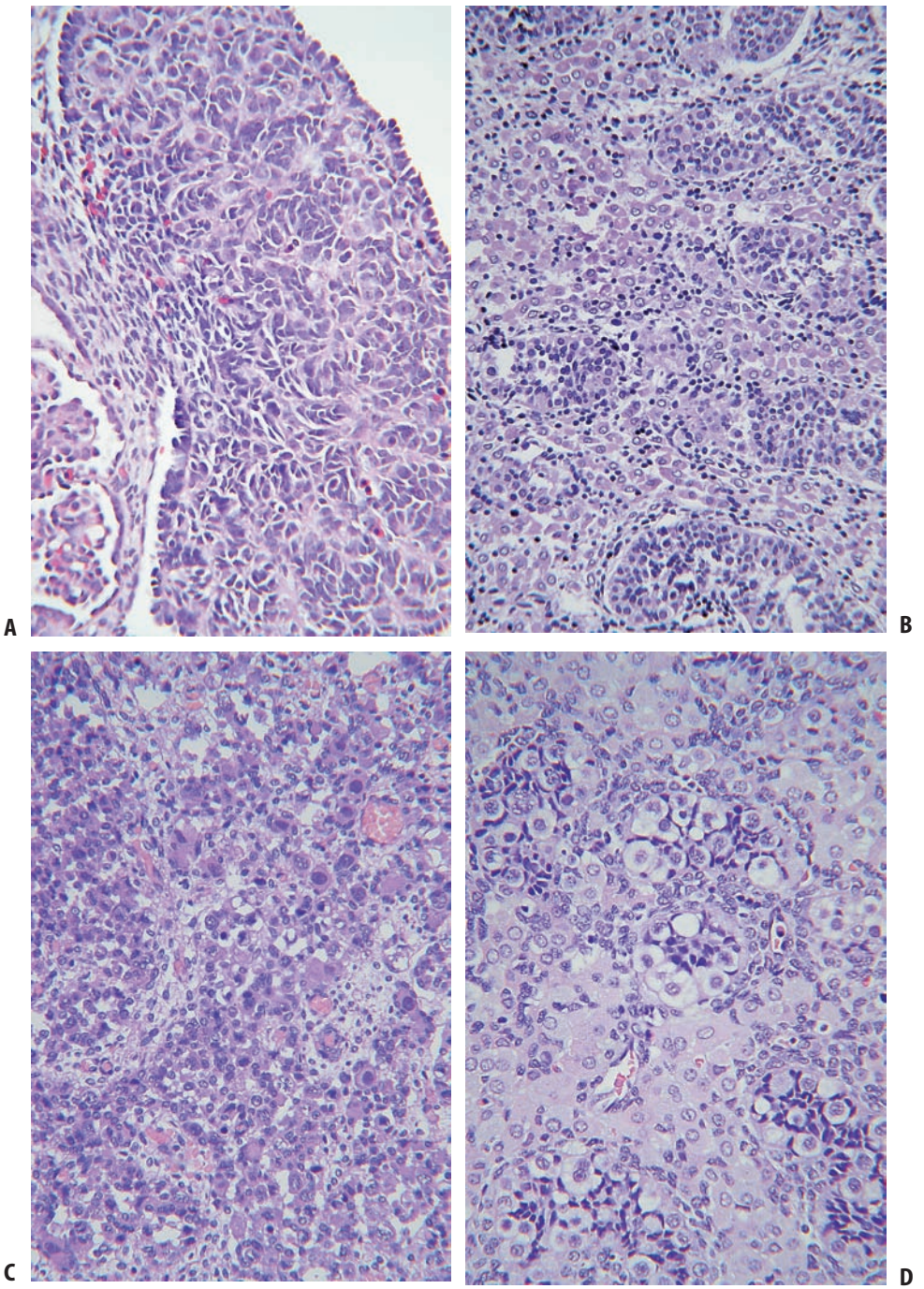

Figure 23.1. Histology of fetal gonads. (A) Indifferent gonad from an embryo at 5 weeks' gestation. [Hematoxylin and eosin (H&E), × 250.] (B) Normal testis from a fetus at 18 weeks gestational age, with abundance of Leydig cells, and seminiferous tubules containing immature Sertoli cells and germ cells. (H&E, × 250.) (C) Normal ovary from a fetus at 18 weeks of gestational age. The oocytes have entered the first meiotic division. (H&E, × 250.) (D) Dysgenetic testis from a 21-week-old fetus with 69,XXY karyotype. The germ cells are abnormally distributed, their nuclei are enlarged, and the Leydig cells are very abundant. (H&E, × 400.)

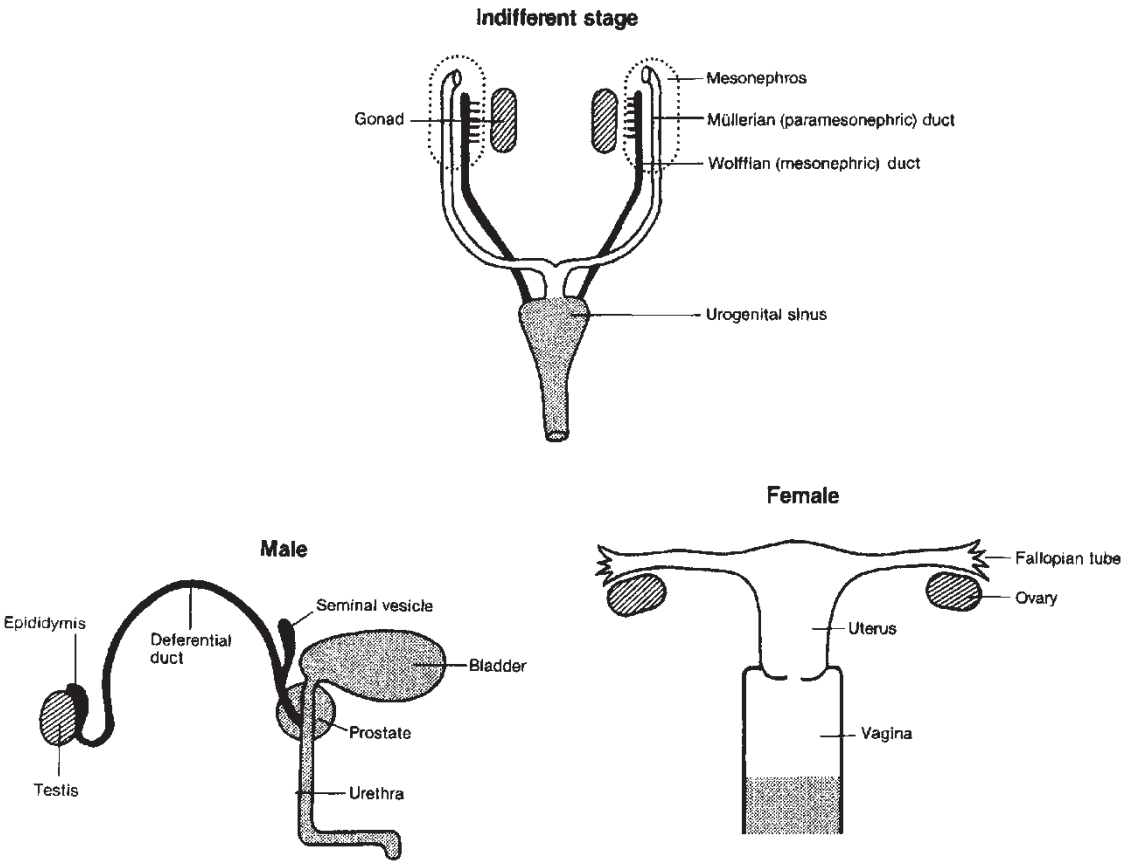

FIGURE 23.2. Differentiation of the internal genitalia. The indifferent stage has paired müllerian and wolffian ducts, and a urogenital sinus. In the male the wolffian ducts have developed into epididymis, deferential ducts, and seminal vesicles. The urogenital sinus has become the prostate and urethra. The müllerian ducts have regressed leaving only the appendix testis (not shown). After female differentiation the müllerian ducts have developed into paired fallopian tubes, uterus, and upper vagina, whereas the wolffian ducts have regressed. Possible remnants of the wolffian ducts may be identified as epoophoron, paroophoron, and Gartner's duct. The urogenital sinus has become the lower vagina and the urethra (not shown).

from the celomic surface epithelium surround the germ cells to form single-layered primordial follicles and later multilayered primary follicles. All germ cells rest in the first meiotic prophase until further differentiation after puberty or until follicular involution.

For a long time it was believed that ovarian differentiation is passive in the absence of testicular differentiation genes. However, duplication of DSS (dosage sensitive sex-reversal), an area on the short arm of the X chromosome, has been shown to cause not only disturbed testicular differentiation but also development of ovarian-like structures in XY individuals. The gene in question is *DAX1*, as its duplication causes development of ovarian-like structures in XY individuals. *Wnt4* is considered essential for the developing ovary, as its expression is downregulated along with testis differentiation (Vainio et al. 1999)

The fetal ovary does not secrete testosterone and AMH. This results in involution of the wolffian ducts and growth of müllerian structures and external genitalia with female characteristics (Figs. 23.2 and 23.3).

Ducts and External Genitalia

In both genetic sexes, the wolffian ducts appear at about 4 weeks' gestation. Under the influence of

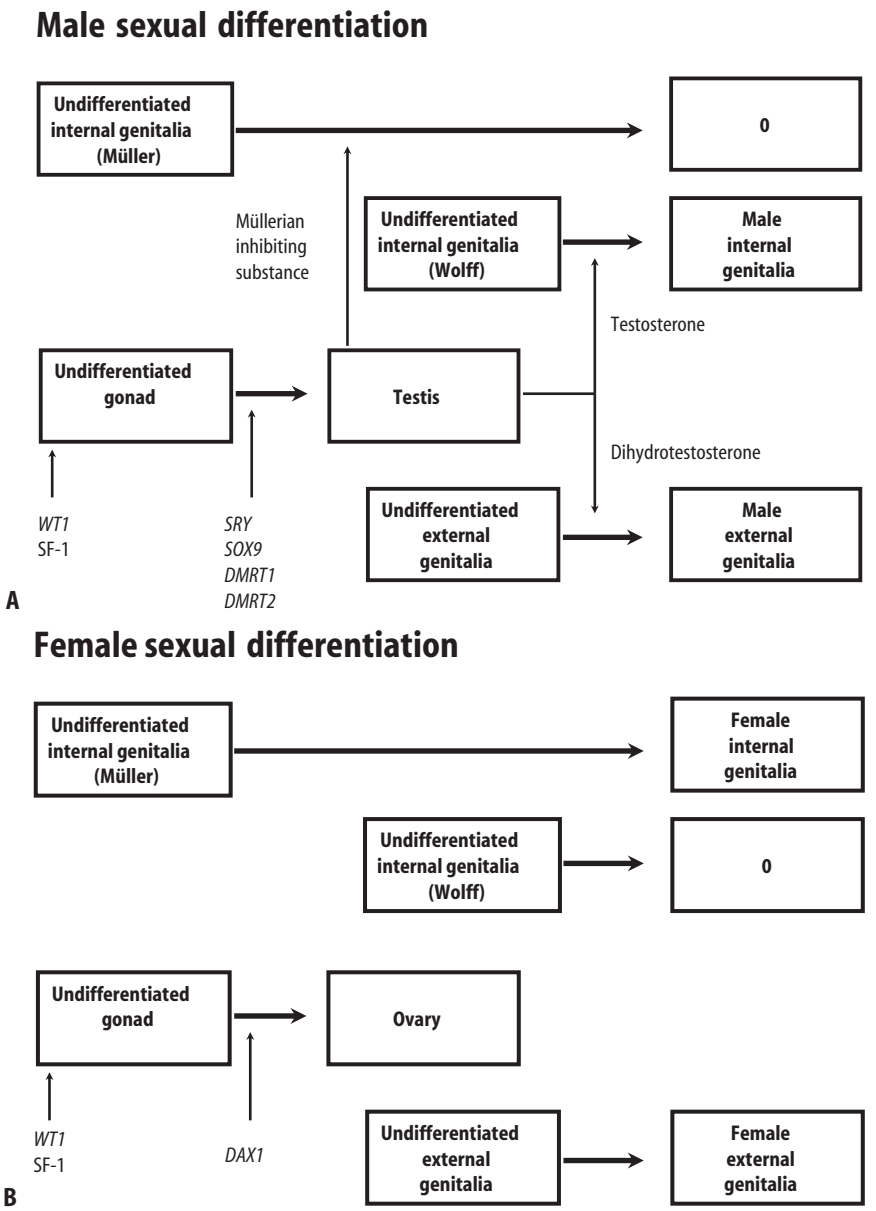

Figure 23.3. Flow charts on sexual differentiation. The *WT1* and SF-1 are involved in formation of the undifferentiated gonad. (A) Male sexual differentiation. The presence of *SRY* and *SOX9* causes the undifferentiated gonad to develop into a testis. The fetal testis secretes two hormones: testosterone and müllerian inhibiting substance. Testosterone influences the wolffian ducts to become epididymis, deferential ducts, and seminal vesicles. The testosterone metabolite dihydrotestosterone is required for development of the male external genitalia including differentiation of the urethra and the prostate. Müllerian inhibiting substance secreted by the fetal Sertoli cells prevents development of the müllerian ducts indicated by 0 in the box. (B) Female sexual differentiation. In the absence of *SRY* and under influence of the *DAX1* gene on the X chromosome the undifferentiated gonad develops into an ovary. The ovary has no significant hormone production during fetal life. Therefore, the müllerian ducts develops into fallopian tubes, uterus, and vagina. In the absence of androgens, the wolffian ducts regresses indicated by 0 in the box, and the external genitalia becomes female in appearance.

testosterone secreted by the fetal testes, the wolffian ducts differentiate into the epididymis, the deferential ducts, and the seminal vesicles. The prostate develops from the urogenital sinus. The müllerian (paramesonephric) ducts, which later develop into female internal reproductive organs, appear in both sexes at about 6 weeks' gestation. In the male fetus, regression of the müllerian ducts induced by AMH begins at 8 weeks' gestation. Müllerian ducts express *Wnt4*, which is also required for renal development (Vainio et al. 1999). In the female, the absence of testosterone causes involution of the wolffian ducts, and the müllerian ducts, in the absence of AMH, develop into the fallopian tubes, the uterus, and the upper third of the vagina (Figs. 23.2 and 23.3).

During the first 2 months of embryonic life, external genitalia are identical in both sexes. They consist of the genital tubercle, the paired lateral labioscrotal folds, and the urogenital sinus (Fig. 23.4). Masculinization of the external genitalia takes place during 9 to 14 weeks' gestation under the influence of testosterone and its 5α-reduced metabolite dihydrotestosterone. The conversion of testosterone to dihydrotestosterone is an important step in male sexual differentiation. Males with 5α-reductase deficiency are born with normal testes but ambiguous external genitalia, that is, a small phallus and persistent urogenital sinus.

The mechanism of testicular descent has not yet been fully elucidated. Both the gubernaculum and epididymis play an important role, and at least three testicular hormones—testosterone, AMH, and, more recently, INSL3 (RLF)—have been implicated in the hormonal control of the testicular descent (Hutson et al. 1997; Toppari et al. 2001). The transabdominal movement of the testis occurs until the first 12 weeks' gestation, at which time the gonads are located close to the internal inguinal ring. Testosterone is required for the involution of the cranial suspensory ligament and migration of the testis through the inguinal canal to the scrotum (Emmen et al. 1998), which starts during the 7th and 8th month of gestation, and in most boys the process is completed at term. INSL3 stimulates the development of gubernacula, which is required to guide descending testes to the scrotum (Nef and Parada 1999; Zimmermann et al. 1999).

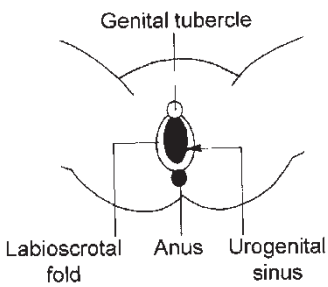

FIGURE 23.4. Indifferent stage of external genitalia at 8 weeks of gestation.

Pathology

Errors of Primary Sexual Determination and Differentiation

These disorders include various forms of defective gonad formation, so-called gonadal dysgenesis, caused by relatively common aberrations of sex chromosomes or rare mutations on genes controlling gonadal development and differentiation (Forest 2001). Dysgenetic gonads have markedly reduced numbers of germ cells, abnormal distribution of the cells, and increased amount of stromal tissue (Fig. 23.1D), frequently leading to streak gonads, sometimes with gonadoblastoma or dysgerminoma.

Turner's Syndrome

Individuals with 45,X karyotype or variants with a fragment of the Y chromosome present has an incidence of 0.5 per 1000 liveborn girls (Nielsen and Wohlert 1991). The gonads in Turner fetuses develop normally until 14 to 16 weeks' gestation and the newborns have normal female genitalia, but have dysgenetic ovaries. From mid-gestation germ cells degenerate rapidly, and the ovaries are gradually replaced by connective tissue (streak gonads). In most Turner girls, only a few follicles are left at birth, but a wide variation exists, and 5% to 10% have spontaneous puberty.

Mixed Gonadal Dysgenesis

This syndrome is a result of chromosomal mosaicism such as 45,X/46,XY and comprises the whole spectrum of external genital development ranging from a normal male to a normal female phenotype, including also Turner stigmata (Forest 2001). Prenatal screening studies suggest that approximately 95% of these individuals have a normal male phenotype; however, most of the patients referred postnatally are females. The histology of the gonads may also vary from typical testicular appearance to that of dysgenetic ovaries. Individuals with a female phenotype or ambiguous genitalia and a karyotype including a Y chromosome are at increased risk of developing germ cell malignancies. Carcinoma-in-situ (CIS) of the testis, a common precursor of germ cell tumors (GCTs) of the adolescent and young-adult type, and gonadoblastoma may be present already in newborns. The fetal dysgenetic gonad may contain areas resembling gonadoblastoma.

Klinefelter's Syndrome

47,XXY and variant karyotypes were found in 1.7 of 1000 liveborn Danish boys, and 47,XYY karyotype with a frequency of 1.1 per 1000 liveborn boys (Nielsen and Wohlert 1991). Testicular histology in fetuses and newborns with Klinefelter's syndrome is qualitatively normal, but degeneration of germ cells has already begun and dramatically accelerates around the onset of puberty (Wikström et al. 2004).

Other Rare Genetic Abnormalities

Abnormal pairing of the X and the Y chromosomes may result in either a 46,XX phenotypic male containing the *SRY* gene of the Y on one X chromosome, or a 46,XY phenotypic female without this DNA sequence. However, a small proportion of XX males are *SRY*-negative. The majority of 46,XX males have normal male phenotype at birth, but *SRY*-negative cases may have hypospadias, cryptorchidism, or other anomalies.

46,XY females (pure gonadal dysgenesis, Swyer syndrome) have normal internal and external female genitalia with the exception of dysgenetic gonads, in which germ cells do not differentiate into oogonia and do not enter the first meiotic division. Thus, primordial follicles are not formed, and it may be impossible to classify the gonad as either a testis or an ovary. 46,XY gonadal dysgenesis may be a result of a mutation in the *SRY* gene.

Male-to-female sex reversal can in rare cases be caused by a partial duplication of the short arm of the X chromosome and ensuing increased dosage of the dosage-sensitive sex locus (DSS), later identified as *DAX1* (Goodfellow and Camerino 1999).

Sex reversal may also be associated with mutations in autosomal genes. Mutations in *WT1* may cause progressive nephropathy (in some cases with Wilms' tumor) and gonadal dysgenesis in both 46,XX and 46,XY individuals (Denys–Drash syndrome). Males are usually born undervirilized, and a diaphragmatic hernia and severe hypospadias are not uncommon. Two phenotypically related but distinct disorders are Fraser syndrome, caused by a mutation resulting in an aberrant ratio of splice isoforms of the WT1 protein (Barbaux et al. 1997), and the WAGR syndrome, which comprises Wilms' tumor, aniridia, ambiguous genitalia, and mental retardation, caused by a mutation in a gene located near *WT1* in 11p13.

Mutations within the *SOX9* gene cause camptomelic dysplasia, a very severe multisystemic disorder with characteristic skeletal defects and male-to-female sex reversal. Around 66% to 75% of 46,XY individuals with camptomelic dysplasia are phenotypic females with gonadal dysgenesis. *SOX9* duplication can cause female-to male sex reversal in 46,XX individuals in the absence of *SRY* (Huang et al. 1999).

True hermaphrodites are extremely rare and have both well-developed testicular and ovarian tissue with germ cells in both compartments. Internal and external genitalia may have all combinations of male and female appearance (Forest 2001). Underlying genetic mechanisms have not yet been elucidated.

Autosomal chromosomal disorders are rarely accompanied by malformations of the genitalia. However, in trisomy 13 syndrome, malformations of the müllerian duct system, abnormal ovarian morphology, cryptorchidism, and hypoplasia of the external genitalia are frequent findings, and abnormal testicular germ cells have been reported

in fetuses with trisomy 21 syndrome (Satge et al. 1996).

Abnormal Virilization

Undervirilized Male

Undervirilization of the male (formerly: male pseudohermaphroditism) may be a result of impaired gonadotropin action, absent or defective androgen biosynthesis or signaling, and impaired AMH production or function (Forest 2001). In the absence of testosterone, the external genitalia feminize. The phallus remains small and bends ventrally to become the clitoris, and the urethral groove remains open. In most cases undervirilization is partial and creates a spectrum of sexual ambiguity with frequent occurrence of maldescended testes and hypospadias. The position of an undescended testis (cryptorchidism) may be abdominal, inguinal, suprascrotal, or high scrotal. Hypospadias is a condition with an abnormal position of the urethral meatus, and may be graded according to increasing severity as glandular, coronal, penile, penoscrotal, scrotal, or perineal. Glandular and coronal formas are the commonest.

The majority of cases with mild genital malformations are observed without any demonstrable endocrine or genetic abnormality. These cases are usually multifactorial and have a strong environmental component, demonstrated by the increasing incidence of cryptorchidism and hypospadias, but with geographic variability (Toppari et al. 2001). In a large study, cryptorchidism was found in 5.9% of newborn boys (Jackson 1988). In boys born before 37 weeks' gestation, the frequency of cryptorchidism is higher. A striking example was provided by recent studies of large cohorts of newborns in, Denmark and Finland. Prevalence of cryptorchidism at birth was 9% in Denmark and 2.4% in Finland, and the rate in Denmark was significantly higher than that reported 40 years earlier (Boisen et al. 2004). Hypospadias was also much more frequent among Danish newborn boys (0.75%) than in Finland (0.27%) ($p < .05$) and more frequent than previously suspected from public registries (Virtanen et al. 2001; Boisen et al. 2005).

Congenital Leydig cell hypoplasia or agenesis impairs testicular hormone production and is a rare cause of poor testicular development and maldescent, with a range of genital ambiguity at birth. Müllerian derivatives are regressed. Testicular histology shows normally formed cords and Sertoli cells but no mature Leydig cells. The lack of Leydig cell differentiation is caused by a mutation in the *LHCGR* gene encoding the LH receptor (Kremer et al. 1995). Similar phenotype of incomplete virilization can be caused by other disorders of testosterone biosynthesis, which may be caused by a mutation in any of the enzymes necessary for this process. If the genetic disorder affects one of the three enzymes that are also involved in cortisol synthesis pathway, pseudohermaphroditism is accompanied by congenital adrenal hyperplasia, which may be manifested by life-threatening salt loss in early infancy. Incomplete masculinization of the external genitalia may also be a result of impaired testosterone metabolism. The most common disorder is steroid 5α-reductase deficiency, the enzyme converting testosterone to dihydrotestosterone. The most frequent phenotype is an external female (usually with enlarged clitoris) but with internal male genitalia and gonads.

Male undervirilization may also be caused by a mutation in the androgen receptor. This disorder is termed androgen insensitivity syndrome or testicular feminization syndrome (Quigley et al. 1995). In its complete form a genetic male embryo develops into a female individual with nondescended but otherwise normal testes, no müllerian duct derivatives, and female external genitalia. In the incomplete form of androgen insensitivity syndrome the phenotype may range from that of a slightly virilized female to that of a normal male. In most cases the testicular histology is unremarkable in the fetus and the newborn, except for an increased proportion of undifferentiated gonocytes, which elevates the risk of developing CIS and GCTs later in life (Hannema et al. 2006).

Virilized Female

Virilization of the female (formerly: female pseudohermaphroditism) is most commonly related to congenital adrenal hyperplasia, which is described in Chapter 24 p. 673. In 46,XX

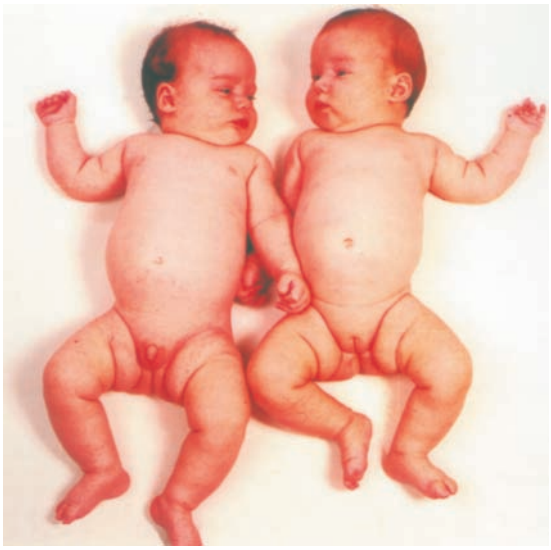

Figure 23.5. A pair of dizygous newborn twins, both with the karyotype 46,XX. The twin to the left is completely virilized and has congenital adrenal hyperplasia. Penis is of normal size, the urethral orifice is normally located, but the scrotum is empty. The twin to the right is a normal female.

individuals with congenital adrenal hyperplasia the phenotype may be that of a normal male with undescended testes (Fig. 23.5).

Other Genital Abnormalities

Male

Damage to the scrotum and the testis may occur after breech delivery, which may cause necrosis of testicular parenchyma and cause subsequent impairment of fertility.

Agonadism, a rare disorder characterized by the absence of testes at birth or by the presence of rudimentary testicular remnants in boys with 46,XY karyotype and various degrees of genital ambiguity at birth has been described. In some patients, especially with familial occurrence, other severe malformations may be present. This anomaly is thought to be a result of testicular regression after the male sex differentiation cascade has been triggered (Forest 2001).

Epispadias is rare. In these cases, the external urethral orifice is located on the dorsal side of the penis, and the malformation is often combined with a defect in the abdominal wall and with bladder exstrophy.

Female

Various abnormalities in the fusion of the paired müllerian structures may be found as isolated phenomena (Fig. 23.6). Malformations of the internal or external genitalia may also be part of a complex major urogenital developmental defect including anal, vaginal, and urethral atresia, for example, sirenomelia. Occasionally the cloaca or the urogenital sinus persist due to incomplete formation of the urorectal or vesicovaginal septa. Cystic dilatation of the cloaca or the urogenital sinus and the connected structures may occur because of concomitant atresia of their external openings. In these instances, underlying endocrine or chromosomal disorder is unusual.

Nonneoplastic ovarian follicle cysts are relatively frequent in the perinatal period and are caused by placental hormones.

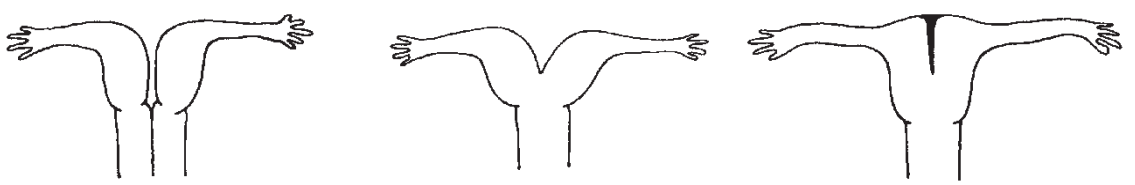

Figure 23.6. Abnormalities of uterus and vagina by incomplete fusion of müllerian ducts.

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24
The Endocrine System

Elizabeth S. Gray

Until recently our knowledge of the fetal endocrine system in the human and its normal development has been limited. We have had even less understanding of how the classical endocrine system with its blood-borne hormones, interacted with the paracrine system and its growth factors. In the last 10 years animal experimentation and epidemiological human studies have led to a dramatic increase in our knowledge and have revealed that the fetal endocrine system is not only pivotal to the fetus but casts exceptionally long shadows into adult life.

It appears that the maturing fetal endocrine system progressively develops feedback and regulatory mechanisms similar to those of the adult. However, the fetal endocrine system also has to incorporate an additional endocrine organ in the placenta, the steroidogenic zone in the adrenal cortex (fetal zone), and a contribution from the central nervous system, which in the fetus is probably capable both of the production of certain tropic hormones and of being a target organ for certain steroid hormones produced by the gonads, adrenal gland, and placenta.

This complex endocrine system is developing and functioning within an organism in which cells of target organs are rapidly reduplicating, differentiating, and maturing under the influence of tissue growth factors (the paracrine system). Thus, the effect of any fetal endocrine disorder is particularly far-reaching; for example, fetal hyperadrenalism can cause gonadal intersex and possibly even alter sexual proclivity in adult life.

In the classic endocrine system specialized cells produce blood-borne hormones that act on distant target cells. They may be proteins, polypeptides, amino acids, steroids, or amines. They are produced by endocrine glands—hypothalamus, adrenal, pituitary, thyroid, parathyroid, and pancreatic islets.

In the paracrine system a wide variety of cells produce peptides called tissue growth factors that act locally on adjacent cells (paracrine) or on secreting cells (autocrine). These peptides increase cell growth and stimulate protein and DNA and RNA synthesis through specific receptors on the target cell surface. Most fetal cells produce growth factors and have many more receptors than equivalent adult cells (Petraglia et al. 1990). These growth factors, structurally similar to viral oncogenes, are important to the growth, differentiation, and modeling of fetal tissues (Han 1996). In this context insulin-like growth factors (IGFs) are particularly important. In animal experiments knockout of IGF type 1 leads to severe growth restriction, whereas overexpression of the *igf2* gene causes fetal overgrowth (Efstratiadis 1998).

There is constant interplay between the two hormone systems, with the classical hormones (particularly thyroxine, insulin, and glucocorticoids) acting directly on tissue IGF gene expression to modulate IGF production (Fowden 2003) and to control the activity and numbers of both IGFs and IGF receptor sites. By these means, hormones control cell growth and differentiation and adapt these to both normal developmental progress and to adverse intrauterine conditions.

Hormones in the fetus are also produced by the placenta, which secretes hormones into both the maternal and fetal circulations (vide infra).

Another source of hormones are those maternal hormones that cross the placenta—thyroxine and cortisol (insulin does not). Because cortisol has powerful catabolic effects on the fetus, the placenta has adaptive mechanisms to control cortisol transfer.

Fetal Hormones and Fetal Growth

Although growth hormone (GH) is essential for postnatal growth, it has long been recognized that it has little importance in intrauterine growth, a fact substantiated by the normal birth weights achieved by infants born with GH deficiency. It is only recently that the interaction of fetal hormones with genes, growth factors, and cell growth factor receptors has been worked out. For those wishing to find out more, there are two excellent reviews of endocrine mechanisms of intrauterine programming (Fowden and Forhead 2004; Murphy et al. 2006).

If there are adverse environmental factors for the fetus, changes occur in the hormone milieu. In poor nutrition the levels of anabolic hormones—insulin, IGF-1, and thyroxine (T_4)—fall, and there is a rise in the catabolic hormones—cortisol and catecholamines. Nutritional plenty, as in gestational diabetes, produces the opposite effect. Specific hormones produce specific changes in fetal growth, for example, an increase in glucocorticoids causes an asymmetric intrauterine growth restriction (IUGR) and resetting, sometimes permanently, of the fetal hypothalamic-pituitary–adrenal axis (HPA). Insulin deficiency produces a symmetric type of IUGR, but with little developmental tissue damage. This is in contrast to the symmetric IUGR of low fetal thyroxine (from fetal and maternal deficiency) in which there are also developmental failures in a range of organs including the central nervous system (CNS). Animal experiments of maternal undernutrition show that these hormone-mediated adaptations of fetal tissue and organ growth have a detrimental affect on the health of the offspring during adult life, the nature of which is directly related to the timing and duration of the antenatal insult. This was confirmed in humans by follow-up of the adult health of those fetuses who survived maternal starvation during the Dutch starvation winter of 1944–45. Likewise epidemiological studies from Britain showed that low birth weight babies were at increased risk of developing a range of degenerative disorders in adult life (Barker and Bagby 2005), and Phillips et al. (1994) showed an increased risk of type 2 diabetes in babies with asymmetric IUGR. Recent studies are now indicating that excess maternal stress during pregnancy causes permanent, sex-related changes in the human HPA (Jones et al. 2006).

Fetal Hormones and the Placenta

The placenta is an important endocrine organ that produces estrogen, progesterone, human chorionic gonadotrophin (hCG), human growth hormone variant (hGHv), and human placental lactogen (HPL). Human placental lactogen probably promotes early embryonic growth (Karabulet et al. 2001). The placenta also produces IGF-1 and IGF-2. Through steroid interconversion activity the placenta controls fetal exposure to endogenous maternal glucocorticoids. This placental regulation of glucocorticoids is a major placental function, because it protects the fetus from these powerful catabolic hormones that reset the fetal HPA axis, alter tissue differentiation, reduce fetal growth, and create long-term detrimental sequelae.

In humans, endogenous maternal cortisol levels are five to 10 times higher than in the fetus (Gitau et al. 1998), producing a steep transplacental gradient. This gradient is maintained by the activity of placental 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), which converts active cortisone to inactive corticosterone (Brown et al. 1993) and vice versa. (It is not capable of metabolizing artificial steroids, which is why therapeutic betamethasone, used to increase fetal lung maturity, can reach the fetus.) In later pregnancy, there is a decrease in placental 11βHSD2 (Sampath-Kumar et al. 1998), which may be a mechanism to allow increased transplacental passage of maternal cortisol and thus accelerate tissue maturation and prepare the fetus for delivery (Murphy and Clifton 2003).

In poor fetal growth, the placental 11βHSD2 is generally reduced and is associated with increased fetal cortisol levels. Experimentally, placental

levels of 11βHSD2 are directly related to levels of oxygen (Hardy and Yang 2002), and increased levels of environmental catecholamines also reduce 11βHSD2 (Sarkar et al. 2001). Both of these could explain why maternal hypoxia and prenatal stress can produce altered fetal glucocorticoid levels and fetal development.

Placental hormones, IGFs and insulin, also control the passage of maternal glucose to the fetus. Glucose is a major fetal nutrient and of entirely maternal origin. Fetal glucocorticoids can act directly on the placental *GLUT1* gene to alter its expression and reset the rate of transfer of maternal glucose with major implications for general fetal growth (Langdown and Sugden 2001).

Finally the placenta cooperates with the fetal zone (FZ) of the adrenal gland cortex to produce progesterone and estrogen. This is carried out by 3β-hydroxysteroid dehydrogenase (3βHSD). The progesterone is used as a substrate by the FZ to produce glucocorticoids. Both progesterone and estrogen are secreted into the maternal circulation to effect maternal adaptation to pregnancy.

A brief outline of the development and function of the fetal endocrine system follows, together with an account of those abnormalities presenting in the neonatal period. Certain clinical conditions, for example, neonatal hypocalcemia, which may mimic endocrine disease, are briefly discussed.

Pituitary–Hypothalamic Axis

Ontogeny

The hypothalamus develops from a part of the diencephalon bulging into the third ventricle. Proliferation of neuroblasts in this area gives rise to the endocrine cells of the hypothalamus, those in the caudal region extending into the infundibulum, and neurohypophysis to become modified neuroglia called pituicytes. The adenohypophysis is formed by ectoderm migrating upward during the 3rd week of gestation (Rathke's pouch). The pituitary portal system, which forms the vascular connection between the hypothalamus and the adenohypophysis, develops from mesenchymal cells beside Rathke's pouch at about 7 to 8 weeks' gestation but is probably not fully functional until 18 to 20 weeks (Goodyer et al. 1979).

The intermediate lobe is poorly developed in humans and arises from the posterior portion of Rathke's pouch (pars intermedia). In the fetus it is more prominent than in the adult (Stark and Frantz 1983).

Hormone Production and Regulation

Although the hypothalamic–pituitary axis is not a functional unit until mid-gestation, individual hormones can be detected much earlier, at about 8 to 12 weeks. The hypothalamus secretes three types of hormones: monoamine neurotransmitters such as dopamine, noradrenaline, and serotonin; the peptides, oxytocin and vasopressin, which are transported to the pituicytes of the neurohypophysis; and those polypeptide hormones acting as releasing factors with the adenohypophysis as their target organ.

The adenohypophysis secretes a range of hormones affecting both specific target organs, for example, adrenal cortex, and metabolically active fetal tissues:

- Growth hormone (GH) is controlled by a releasing factor and by a release-inhibiting factor (somatostatin) from the hypothalamus. Its role in intrauterine growth is minimal: thyroxine, growth factors, insulin, and fetal nutrition are all more important in maintaining fetal growth (vide supra).
- Prolactin (PRL) levels increase rapidly between 30 and 40 weeks' gestation, and then fall after birth, reaching the normal prepubertal range by about 6 weeks of age. The role of prolactin in fetal life is unclear, but it is reduced in the cord blood of infants who go on to develop respiratory distress syndrome (Smith et al. 1979).
- Thyrotrophin (thyroid-stimulating hormone, TSH) is first detectable at 12 to 14 weeks' gestation. It peaks at 20 weeks, by which time the hypothalamic/pituitary/thyroid axis is functional, and it is a prerequisite for fetal thyroid function, as maternal TSH does not cross the placenta. A placental thyrotrophin has been identified but cannot alone support normal fetal thyroid activity. There is a sudden increase

in fetal TSH production at birth and increased thyroxine levels 1 to 2 hours later. This increase occurs after cesarean and vaginal delivery. Histological changes also take place in the thyroid at this time (Sclare 1956).
- Pituitary adrenocorticotropic hormone (ACTH) is detectable from 7 weeks and peaks at 19 weeks' gestation. Although a placental ACTH exists, it appears from studies of the anencephalic fetus (Gray and Abramovich 1980; Young et al. 1989) that an intact hypothalamic pituitary adrenal axis is necessary to support the hyperplastic FZ of the adrenal cortex from 16 weeks, and perhaps even 12 weeks.
- Gonadotropins (follicle-stimulating hormone and luteinizing hormone) are also produced in utero.

The intermediate lobe responds to the stress of parturition by secreting endorphins, which have opiate action (Facchinetti et al. 1989). Fetal melatonin is secreted here. In fetal sheep there is a diurnal rhythm of secretion and also evidence that this daily melatonin rhythm may entrain the daily rhythm of fetal breathing (Houghton et al. 1993). The role of human fetal melatonin may be similar.

Pathology

Developmental Abnormalities of Structure

Isolated pituitary aplasia and hypoplasia, both rare, are due to a defect in the formation of Rathke's pouch, which leads to total or partial absence of the adenohypophysis, but with an intact hypothalamus and neurohypophysis (Moncrieff et al. 1972). These normally grown infants present in the neonatal period with profound hypoglycemia; at necropsy, the pituitary is small or absent. The adrenals are hypoplastic because of poor development of the fetal cortex, and the testes are small with absent Leydig cells. Both ovaries (Fig. 24.1) and thyroid appear inactive. More commonly, deficiency of pituitary function is associated with major cranial and facial deformities, for example, alobar holoprosencephaly (see Chapter 26), facial clefts, congenital cysts of Rathke's pouch (Cornelia de Lange's first syndrome), and anencephaly. Abnormalities of size, shape, and position of the pituitary gland are described in trisomies 18 and 21 (Kjaer et al. 1998a,b)

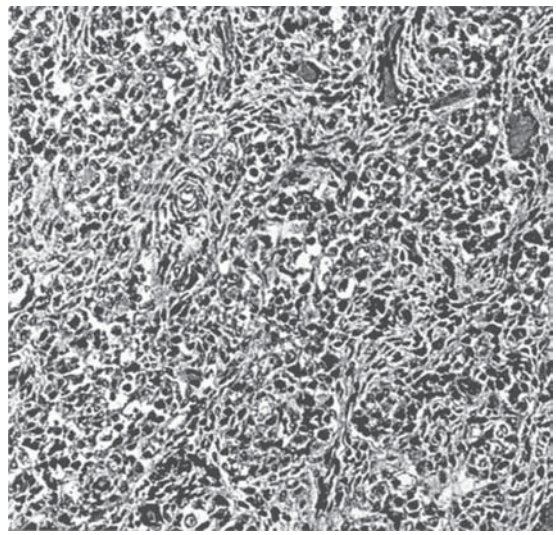


Figure 24.1. Ovary. Neonate, alobar holoprosencephaly. Undifferentiated spindle cell stroma, poor follicle formation. No oocytes. [Hematoxylin and eosin (H&E), ×180]

Anencephaly

Anencephaly is a neural tube defect, with a polygenic inheritance pattern (see Chapter 26) in which the cephalad portion of the neural tube is grossly malformed with absence or disorganization of much of the brain, including the hypothalamus and neurohypophysis.

Malfunction of the hypothalamic–pituitary axis throughout fetal life explains some of the findings in the anencephalic syndrome. Anencephalic pregnancies tend to be either abnormally short or abnormally long (Naeye and Blanc 1971), possibly due to dysfunction of the pituitary–adrenal axis (Karalis et al. 1996). Naeye and Blanc also noted abnormal growth patterns in anencephalic fetuses, with the premature group showing generalized growth restriction. This atrophy affects all organs except lymphoid tissue, the thymus being relatively large—an effect of reduced cortisol production by hypoplastic adrenals. In mature anencephalics, subcutaneous fat is frequently excessive. A similar finding in decapitated rabbit fetuses is due to increased insulin levels and abolished by ACTH (Jack and Milner 1975). Abnormal growth and excess fat have been attributed to a disorder

of GH production, but we now know that IGFs, glucocorticoids, and insulin, not GH, determine intrauterine growth (vide supra). The suggestion that the growth disturbance is due to abnormal insulin production is interesting in view of disturbed carbohydrate handling and excess adiposity in mature anencephalics (Hayek et al. 1973).

The most constant feature in the anencephalic is severe adrenal hypoplasia; the glands weigh only 10% of normal at birth (see Secondary Adrenal Hypoplasia, p. 668). This appears to be due to the failure of the hypothalamic pituitary adrenal axis (HPA axis). Not surprisingly an anencephalic fetus will have normal-sized adrenal glands when it shares its circulation with its non-anencephalic twin who has an intact HPA axis. The thyroid gland and gonads appear normal in anencephaly. The endocrine pancreas is normal, but if the mother is diabetic, it may fail to develop the expected islet hyperplasia (Van Assche et al. 1969).

The relationship between the hypothalamic-pituitary axis and the function of the islet B cells is still not understood. Milner and Hill (1987) describe it as "the pea under the princess's mattress"!

Histological examination of the anencephalic pituitary shows an absent or abnormal hypothalamus and neurohypophysis (Salazar et al. 1969). The adenohypophysis is small with normal cell types. However, electron microscopy and immunocytological examination show that the corticotrophs are degenerate and decreased in number.

Idiopathic Pituitary Insufficiency

The term *idiopathic pituitary insufficiency* is used to describe those cases that have no anatomical or traumatic explanation.

In isolated GH deficiency, birth weight is normal, as intrauterine growth is not GH dependent, and only those infants who develop severe neonatal hypoglycemia present in the neonatal period. In about 3% to 5% of cases it is an inherited condition, usually in autosomal recessive fashion, but autosomal dominant and X-linked recessive patterns are described. Isolated growth hormone deficiency is reported in congenital rubella (Preece et al. 1977) and Turner's syndrome (Faggiano et al. 1975). The anterior pituitary may be involved in congenital cytomegalovirus (CMV) infection (Fig. 24.2); functional impairment does not occur.

Multiple pituitary hormone deficiency is often related to traumatic or breech delivery, and is more likely to present in the neonatal period, sometimes with hypoglycemic convulsions.

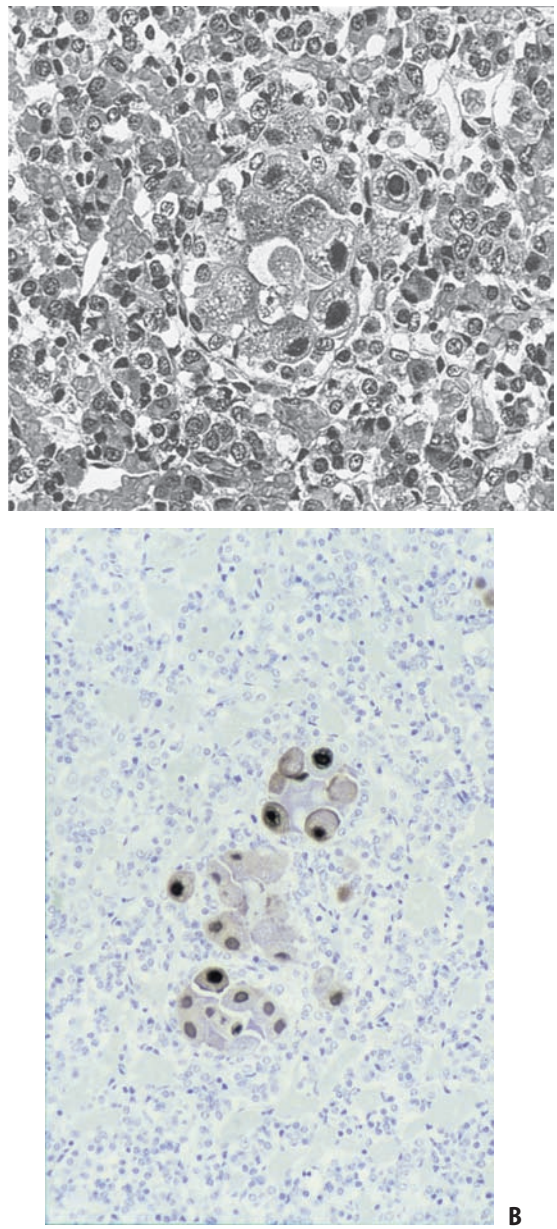


Figure 24.2. Pituitary. Neonate with congenital cytomegalovirus (CMV). CMV inclusions in acinar cells of adenohypophysis. (A: H&E, ×400; B: [peroxidase antiperoxidase], ×280)

Adrenal Glands

Ontogeny

Adrenal glands comprise a medulla around which are layers of cortex. Cells that form the early FZ of the adrenal cortex are first identified at 4+ weeks' gestation, arising from a mesothelial zone medial to the urogenital ridge. By 7 weeks, cells from the neural crest migrate into the center of the early cortex to form the medulla. At about the same time a second migration of cells derived from coelomic epithelium envelop the entire gland. These cells later differentiate into the outer transitional zone of the FZ, and the definitive zone (DZ) or adult zone. During the first trimester the DZ is poorly formed and nonfunctional, while the FZ shows steroidogenic activity from the 7th week; this increases steadily through the first and second trimesters, and the FZ becomes large, comprising 80% of the entire adrenal cortex. From about the 25th week of gestation, the DZ shows thickening and evidence of steroidogenic activity. During the last trimester FZ and DZ are fully developed. The FZ is formed by bulky polygonal cells with abundant eosinophilic cytoplasm, which continues to occupy 70% to 80% of the adrenal cortex until birth, after which it shrinks progressively, comprising only 50% of the gland at 6 weeks of age. Differentiation of the DZ into the zona glomerulosa and zona fasciculata occurs between the 2nd and 4th week of postnatal life. The zona reticularis is slow to appear and is seen between 1 and 4 years of age.

Hormone Production and Regulation of the Adrenal Cortex

Although the DZ is capable of steroidogenesis in the latter half of gestation, it is the FZ, together with the placenta, that secretes the intrauterine steroid complement. Neither the FZ nor the placenta can do this alone, as each lacks certain essential enzymes. In particular, the FZ has low levels of 3βHSD, while the placenta has plenty (Buster 1980). The FZ extracts low-density lipoproteins from fetal blood (Parker et al. 1996) and converts them, via cholesterol to D^5-pregnenolone sulfate, 17-hydroxy-D^5-pregnenolone sulfate, and dehydroepiandrosterone sulfate (DHEA-S) (Simpson et al. 1979). Because of the FZ deficiency of 3βHSD, these three steroids are secreted in large amounts to be used as substrates by 3βHSD in the placenta to synthesize progesterone and estrogen. Progesterones are returned to the fetal circulation where the FZ uses them as substrates for conversion to the glucocorticoids. Placental estrogen is secreted into both fetal and maternal circulations. Although the placenta and adrenal cortex cooperate to produce glucocorticoids, other organs can convert cortisol to cortisone and vice versa. This peripheral steroid interconversion occurs in the placenta, using maternal glucocorticoids, and Strauss et al. (1996) postulate that these tissue steroid interconversions stimulate the development of the fetal hypothalamic-pituitary and adrenal cortex.

Regulation of fetal adrenal steroidogenesis is not fully understood, but is reviewed by Pepe and Albrecht (1990). Study of the fetal adrenal cortex of anencephalics suggests that an intact hypothalamic/pituitary/adrenal axis is necessary for FZ maintenance after about 16 weeks' gestation (Gray and Abramovich 1980). Prior to this age, IGFs produced by the adrenal, and peptides of placental origin, including hCG and a chorionic corticotrophin-like peptide, may be important for development of the fetal adrenal. Various pituitary peptides, particularly from the intermediate lobe, have been suggested as promoters of fetal adrenal function, but ACTH itself causes marked stimulation of fetal cortex in culture (Fujieda et al. 1981). Moreover, in the presence of high levels of estrogen from the placenta, ACTH promotes DHEA-S rather than cortisol production. The resulting low cortisol would then stimulate ACTH production (Fujieda et al. 1982) and cause the typical hyperplasia of the normal FZ. There is a complex interaction between the placental steroid interconversions and various tissue growth factors; for example, epidermal growth factor (EGF) increases 3βHSD activity in the fetal adrenal (Coulter et al. 1996a), and further studies by the same group show that the tropic actions of ACTH are mediated by insulin-like growth factor 2 (IGF-2), which is synthesized by the fetal adrenal cortical cells (Coulter et al. 1996b). Part of the difficulty in studying the function of the FZ has been in isolating a pure culture of FZ cells. Coulter (2004) has, by laser capture microdissection, now

obtained such a culture, so we can expect further elucidation of the FZ role.

At birth, loss of the placenta removes both the inhibitory influence of estrogen and several steroid substrates, causing the FZ to shrink. Plasma ACTH also falls in the first few days of life. Circadian rhythms of ACTH and cortisol secretion are not achieved until 3 to 4 months of age.

The adrenal medulla is formed by neural crest cells, which multiply to form neuroblastic islands. These cells mature and differentiate into catecholamine-secreting cells. The adrenal medulla is the major fetal source of catecholamines, and their production is controlled by glucocorticoids from the adjacent adrenal cortex. Catecholamines increase surfactant production in fetal lung and are probably important, along with thyroxine, for thermogenesis in the neonate (Artal 1980).

Pathology

Developmental Anomalies

Adrenal Agenesis

This condition is extremely rare and, although previously reported in anencephaly, the finding reflects poor dissection. Ectopic adrenals, or ectopic adrenal cortical tissue seen as yellowish nodules is particularly common in organs of the urogenital tract including the testes, ovaries, epididymis.

Secondary Adrenal Hypoplasia

This condition occurs in alobar holoprosencephaly but most commonly in the anencephalic syndrome (see Anencephaly p. 665). In anencephaly the adrenal glands are smaller than normal from

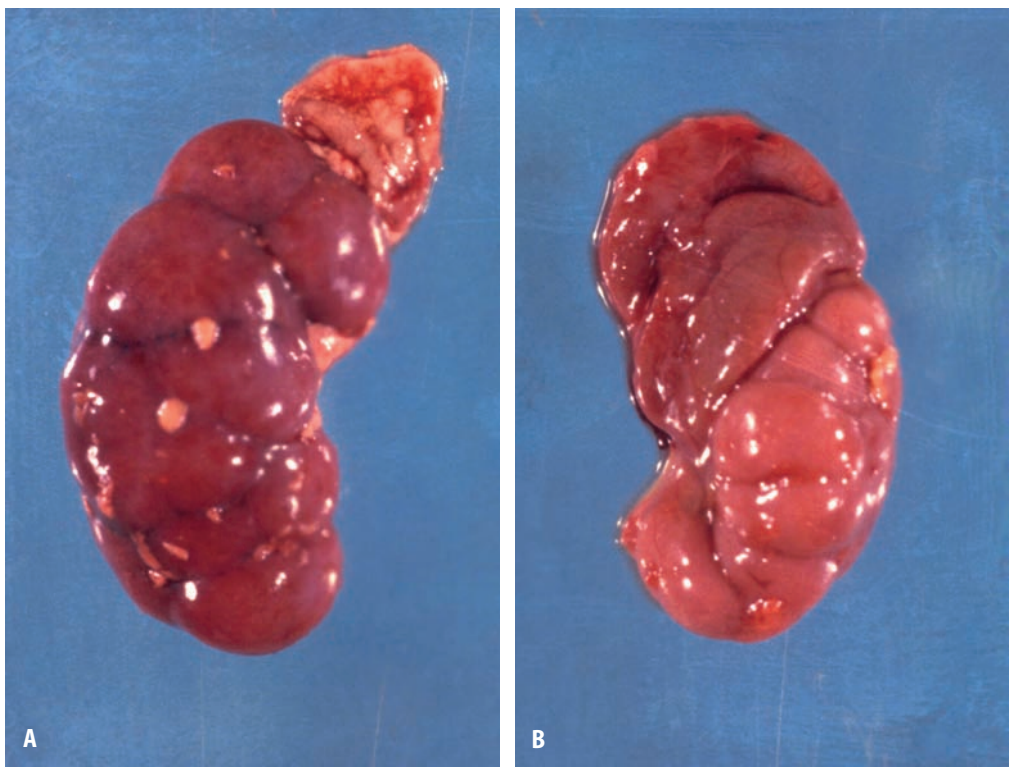


Figure 24.3. Fetal kidney and adrenal gland. (A) Anencephalic fetus, 19 weeks' gestation: small yellowish adult shaped adrenal. (×3) (B) Normal fetus, 18 weeks' gestation: large pink adrenal gland. (×3)

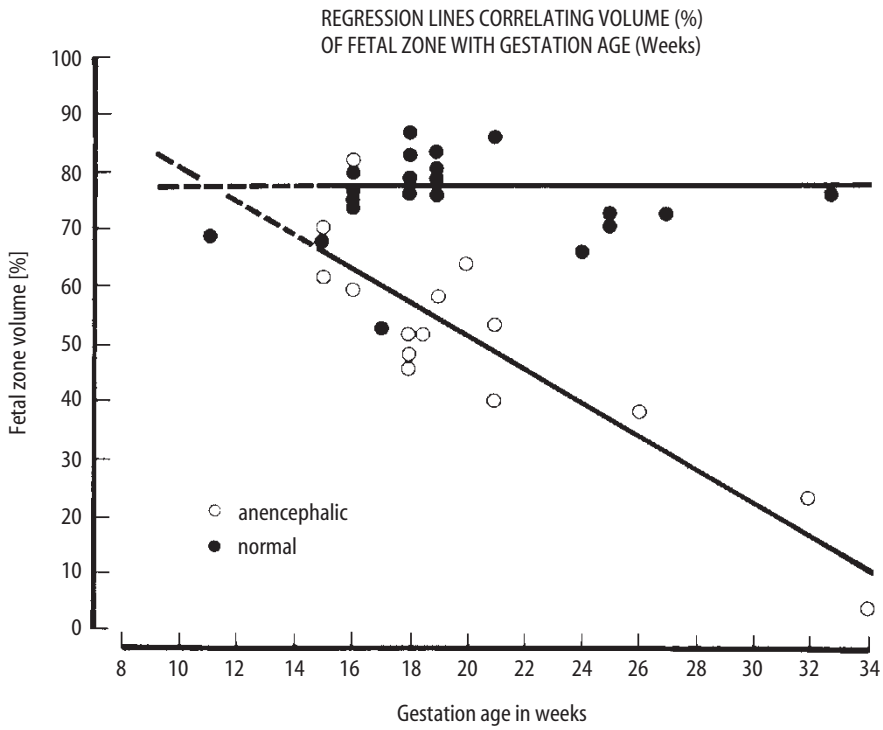


Figure 24.4. There is progressive diminution in the volume of the fetal zone (FZ) cortex in the anencephalic fetus.

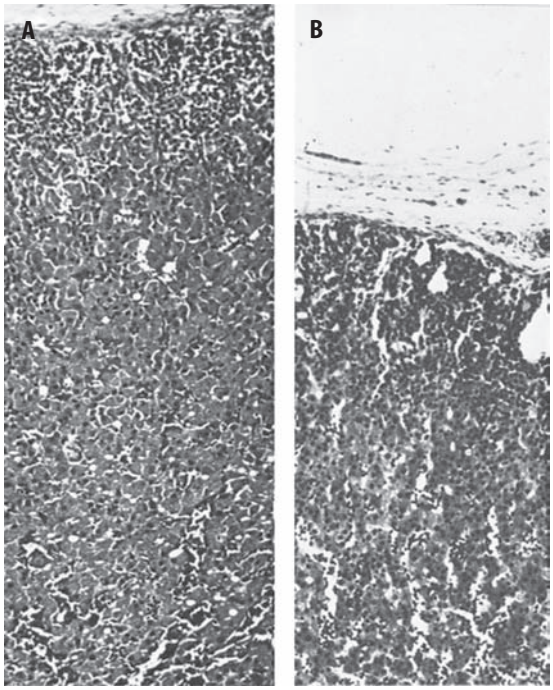


Figure 24.5. Adrenal cortex at 19 weeks' gestation. (A) Normocephalic: FZ is 80% volume. (B) Anencephalic: FZ is 50% volume. (A,B: H&E, ×250)

as early as 16 to 18 weeks' gestation, and in shape come to resemble miniature adult adrenal glands (Fig. 24.3).

At birth they weigh only 10% of the normal combined weight of 9 g. This hypoplasia is due to progressive shrinking (Fig. 24.4) of the FZ of the adrenal cortex (Fig. 24.5) from 80% of cortical volume to a mere 20% of cortical volume by 33 weeks' gestation (Gray and Abramovich 1980).

Idiopathic Adrenal Hypoplasia

This condition exhibits three histological patterns, of which the most common is the "anencephalic" pattern of FZ atrophy. Kerenyi (1961), who described only two patterns, believed that the anencephalic type was always due to a disorder of the pituitary or hypothalamus and therefore not truly idiopathic but secondary. Histology of type 2, cytomegalic adrenal hypoplasia, shows the adrenal cortex to be disorganized with marked cytomegaly of intensely eosinophilic cortical cells (Fig. 24.6); it is not associated with pituitary abnormality and usually shows a recessive X-linked

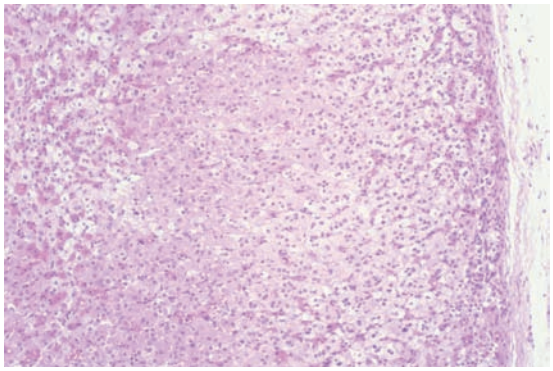


Figure 24.6. Hypoplastic adrenal, male infant, 4 months old. X linked adrenal hypoplasia.

inheritance trait. A mutation in the gene *DAX1* is the cause of the X-linked form of adrenal hypoplasia (Guo et al. 1995) and the frequently associated hypogonadotropic hypogonadism. A third type, where both the FZ and DZ are proportionately reduced, is described by Larroche (1977).

Partial Adrenal Hypoplasia

This condition occurs in some infants with trisomy 18. The glands show a reduction in the width of the FZ. This change in the FZ may be a reflection of the placental hypoplasia and hypofunction found in trisomy 18. A similar type of adrenal hypoplasia is associated with severe intrauterine growth restriction, but its etiology may be different.

Fusion

The adrenal glands are occasionally fused in the midline in thoracolumbar spina bifida.

Acquired Pathology

Adrenal hemorrhages are not uncommon in infants with severe perinatal anoxia. The hemorrhages usually occur in the inner cortex and may be small, or massive and associated with extensive glandular necrosis (Fig. 24.7). Occasionally, hemorrhage may be subcapsular or predominantly periadrenal, spreading into retroperitoneal tissues.

Breech delivery, high birth weight, and prolonged, difficult labor are associated with adrenal hemorrhage, often unilateral. They are also seen in premature infants without obvious birth trauma.

Adrenal calcification can occur in utero following infection, for example, CMV (Fig. 24.8) and

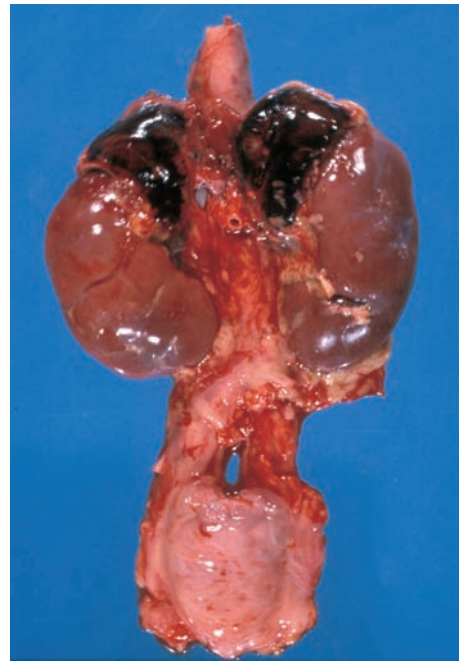


Figure 24.7. Premature (30 weeks) neonate with respiratory distress syndrome. Massive bilateral adrenal hemorrhages.

toxoplasmosis. In such infections, foci of calcification occur in other viscera, particularly liver, myocardium, and brain. The identity of the infecting organism is often not established. Centrally placed microscopic foci of calcification are found

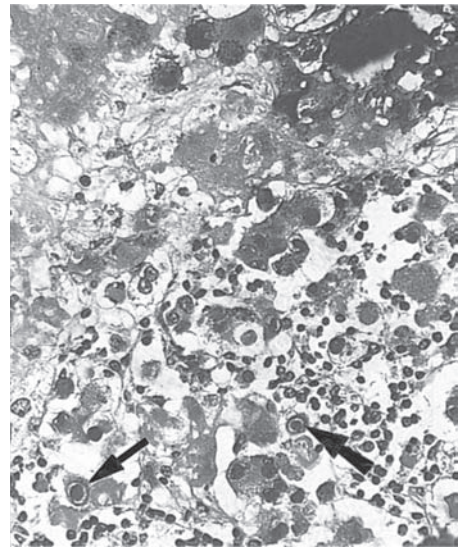


Figure 24.8. Adrenal, neonate. Focal calcification, CMV inclusions (arrows) and lymphocytic infiltrate. (H&E, ×220)

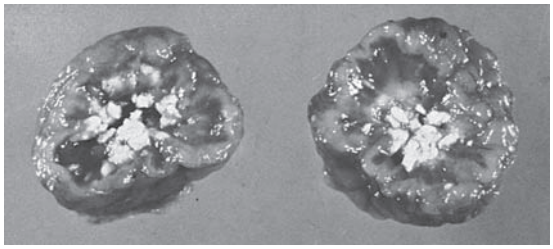


Figure 24.9. Adrenal glands, mulberry-shaped glands with central calcification. Unknown etiology, four affected siblings. (×2)

in the large fatty adrenals (see below) of hydrops fetalis, whether of immunological or nonimmunological type. A similar calcification is found in the lipid-laden glands of Wolman's disease.

Extensive adrenal calcification in neonates and infants is usually the sequel of hemorrhagic necrosis caused by perinatal asphyxia. The calcification is usually in the inner portion of the cortex and the medulla, the outer cortex being well preserved. This dystrophic calcification can occur as early as 9 days posthemorrhage.

We have observed in two unrelated Scottish families with normal parents, four children with congenital nephrotic syndrome (Finnish type) who have also had massive congenital bilateral adrenal calcification. The adrenal glands in the affected children had an unusual mulberry shape (Fig. 24.9). None of the children showed clinical evidence of adrenal dysfunction, and all died in infancy of renal failure.

Fatty Adrenals

The fetal neonatal adrenal is normally pink and fleshy. When the lipid content is increased, the adrenal appears yellow, and on histological examination the FZ cells are clear and vacuolated (Fig. 24.10) and contain much stainable fat. Yellow, lipid-rich adrenals are a striking feature in some hydropic fetuses (Fig. 24.11). Larroche (1977) maintains that lipid-rich adrenals are specific to immunological (i.e., rhesus isoimmunization) hydrops, but we have frequently seen it in nonimmunological hydrops fetalis. Sometimes these adrenals show microscopic foci of calcification. The accumulation of lipid is probably a nonspecific response to prolonged intrauterine stress. Becker and Becker (1976) examined the amount and distribution of fat in the fetal cortex of

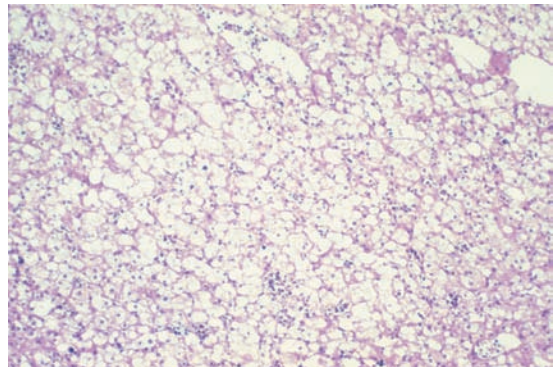


Figure 24.10. Fetal adrenal cortex, immunological hydrops fetalis (26 weeks). Vacuolated lipid-rich cortical cells. (H&E, ×220)

stillborn infants. They describe three distinct histological patterns, each related to the duration of the intrauterine stress.

In Wolman's disease due to acid lipase deficiency, massive amounts of neutral lipid are present in the enlarged, calcified adrenals as well as in the liver and other organs. Deficiency of 20–22 desmolase, an enzyme very early in steroid synthesis, produces an accumulation of both fat and cholesterol in enlarged hyperplastic adrenals.

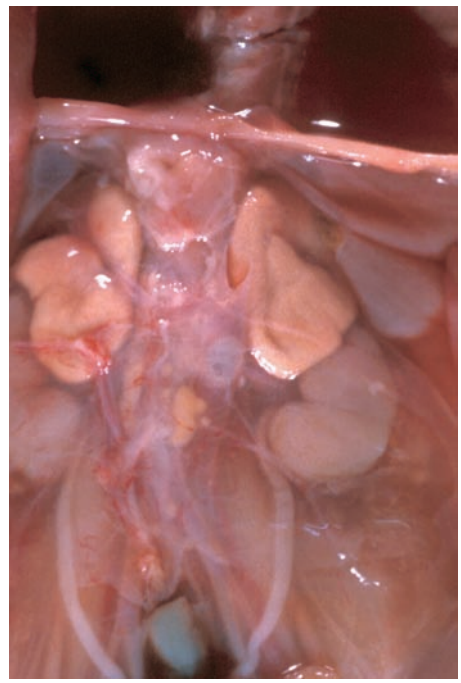


Figure 24.11. Hydrops fetalis (same case as in Figure 24.10). Large pale–yellow adrenals. (×2.3)

Microscopic Pathology

Neuroblastoma In Situ

The adrenal medulla is formed from immature small dark cells called neuroblasts. Nodules of these cells may persist in the center of the gland and be found incidentally on histological examination (Fig. 24.12). Some are quite large aggregates with rosette formation. They are more common in early than late gestation. The majority regress or mature, but some may be the nidus of malignant neuroblastoma (see p. 340). It is suggested that these nests of neuroblasts are more common in the adrenals of anencephalics (Van Hale and Turkel 1979).

Hemopoietic Activity

Foci of hemopoietic activity may be present in the adrenals of infants with no clinical evidence of increased hemopoiesis. In leukemoid reactions (see Chapter 8) the adrenal is frequently infiltrated by cells of the myeloid series.

Adrenal Cytomegaly

A frequent incidental finding at necropsy is adrenal cytomegaly, where some of the eosinophilic cells of the FZ show nuclear gigantism (Fig. 24.13). It is thought to be due to cellular exhaustion following a period of hyperactivity (Fasano and Greco 1996); when severe, it is usually part of the Beckwith-Wiedemann syndrome (Fig. 24.14) (visceromegaly, exomphalos, hyperplasia of the islets of Langerhans, macroglossia, and adrenal cytomegaly). In this condition it may be present as early as 20 weeks' gestation. Adrenal cytomegaly is also found in type 2 idiopathic adrenal hypoplasia but differs in that there is excessive, glassy cytoplasm and normal-sized nuclei (Fig. 24.6).

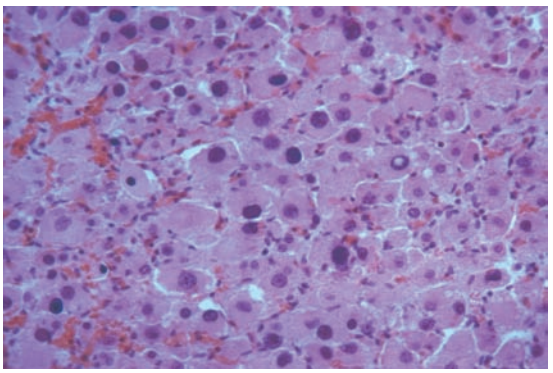


Figure 24.13. Adrenal cortex, normal neonate. Focal nucleomegaly and cytomegaly. (H&E, ×180)

Pseudocysts and Pseudofollicular Changes

Pseudocysts and pseudofollicular changes are commonly found in the subcapsular adult cortex in aborted fetuses and neonatal deaths. The cells are arranged in a gland-like fashion, and eosinophilic fluid is generally present in the lumen (Fig. 24.15). These changes have been interpreted as a response to stress or anoxia (DeSa 1978) and are described in infection, premature rupture of membranes, and following termination of pregnancy (Gaillard et al. 1990).

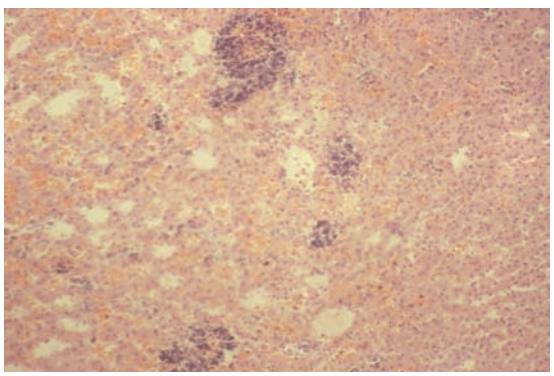


Figure 24.12. Fetal adrenal at 28 weeks' gestation. Numerous aggregates of neuroblasts with some rosette formation. (H&E, ×170)

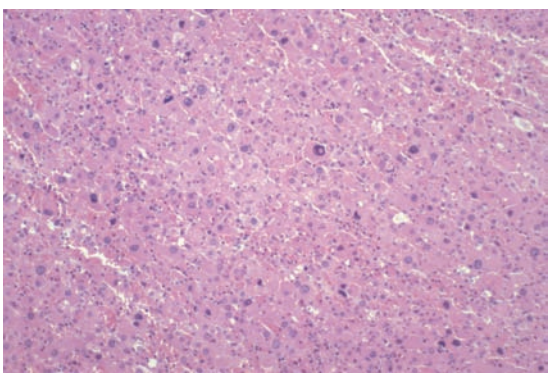


Figure 24.14. Adrenal cortex, Beckwith–Wiedemann syndrome. Diffuse cytomegaly. (H&E, ×140)

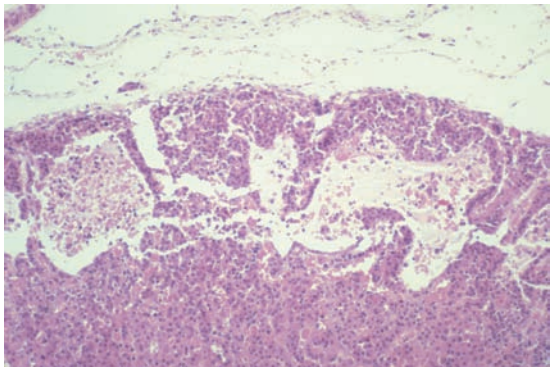


Figure 24.15. Adrenal cortex, neonate (26 weeks) with respiratory distress syndrome. Pseudofollicular cysts in the definitive cortex/zone. (H&E, ×140)

Other Changes

As the result of prolonged stress, other changes occur in the morphology of the adrenal gland. There may be reduction in the large pale FZ cells and an increase in the compact cells. Alternatively, the adrenals may be pale with increased lipid.

Functional Pathology

Congenital Adrenal Hyperplasia (Adrenogenital Syndrome)

Congenital adrenal hyperplasia is caused by an enzyme defect in cortisol synthesis; low cortisol levels cause excessive ACTH secretion. Adrenocorticotropic hormone causes further stimulation of the adrenal cortex, but because of the blocking effect of the deficient enzyme, cortisol levels remain low and ACTH production is constantly switched on. The effect is adrenocortical hyperplasia and increased steroidogenesis with excessive production of androstenedione and thus of testosterone and estradiol. In 90% of cases, the enzyme that is deficient is 21α-hydroxylase. As this enzyme is also necessary for the synthesis of aldosterone, complete deficiency will produce a salt-losing state, which can be fatal in the neonatal period.

If the enzyme defect occurs in a female fetus (80%), virilization with ambiguous genitalia, a female pseudohermaphrodite, is seen (Fig. 24.16). In the male without salt loss, the onset of precocious puberty first alerts clinicians to the possibility of congenital adrenal hyperplasia.

11β-hydroxylase is much less commonly deficient and causes an excess production of aldosterone precursors as well as increased androgens. These infants have cardiomegaly and hypertension as a result of chronic sodium and water retention, in addition to virilization. Because it affects a very early stage in steroid synthesis, 3β-hydroxylase deficiency produces severe, often fatal, salt loss with little virilization.

These enzyme deficiencies are inherited in an autosomal recessive pattern. The adrenals, in all forms, are greatly enlarged (average = 15 g) and have a corrugated "cerebriform" appearance (Fig. 24.17) and a brown cut surface. Histological examination (Fig. 24.18) shows nodular hyperplasia of the cortex with an increase in the active eosinophilic or compact cells that stream outward toward the surface (Fig. 24.19). Lipid-containing cells of the zona fasciculata are greatly reduced.

Infections

In systemic listeriosis, the adrenals are usually involved; white abscesses, the size of pinheads, with a characteristic histology are present (Fig. 24.20). Toxoplasmosis, echovirus (Fig. 24.21), and herpes infections (Fig. 24.22) produce typical

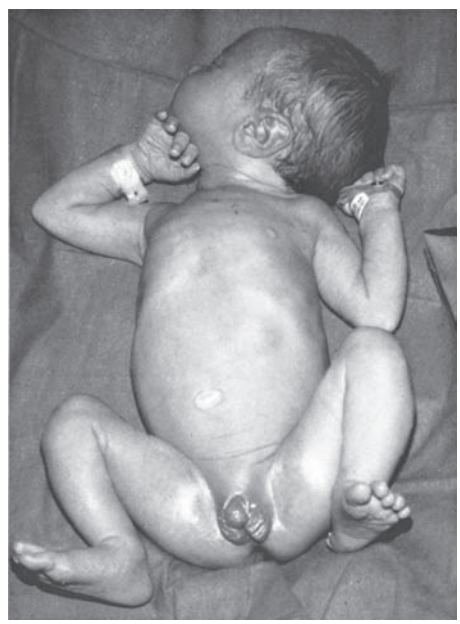


Figure 24.16. Female infant with congenital adrenal hyperplasia. The clitoris is hypertrophied and the labia are rugose.

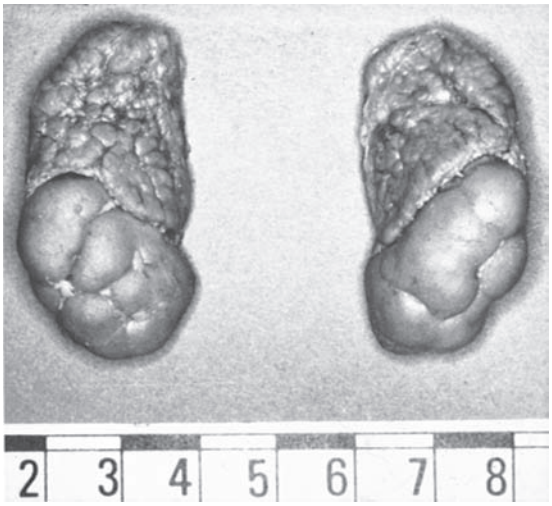


Figure 24.17. Adrenal gland, congenital adrenal hyperplasia. Corrugated cerebriform surface. (Courtesy of P.J. Berry, Bristol, England.) (H&E, ×60)

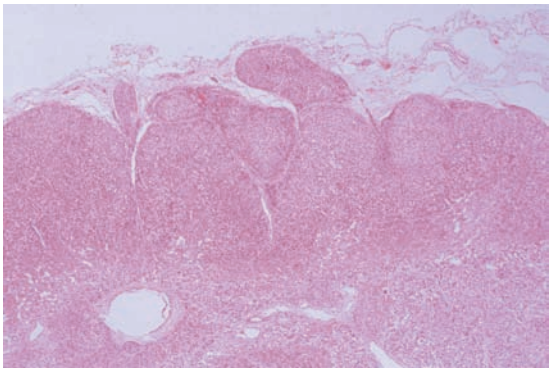


Figure 24.18. Adrenal cortex, congenital adrenal hyperplasia. Irregular nodular hyperplasia of the cortex. (H&E, ×190)

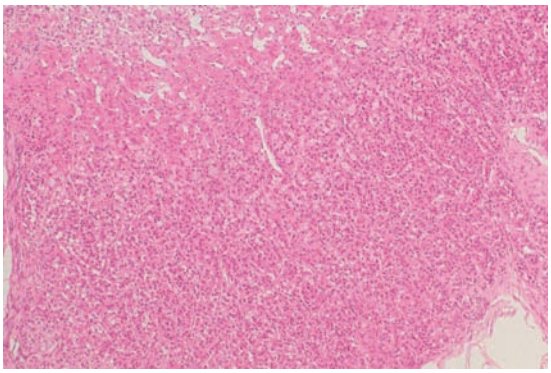


Figure 24.19. Adrenal cortex, neonate (19 days old) with congenital adrenal hyperplasia. Increased compact cells in definitive cortex. FZ cells more eosinophilic than usual and occasional cells showing nucleomegaly. (H&E, ×190)

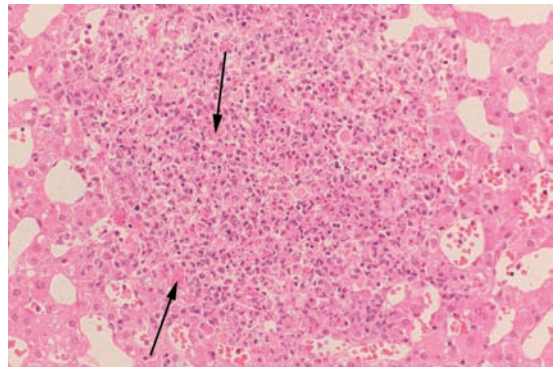


Figure 24.20. Adrenal cortex, neonate (29 weeks' gestation) with generalized listeriosis. Typical "granuloma" with central fibrin (arrows), scanty polymorphs and monocytes. (H&E, ×130)

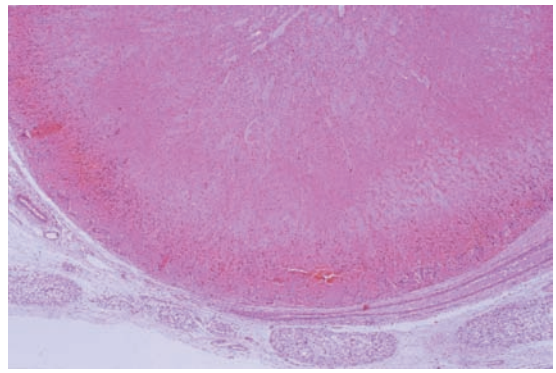


Figure 24.21. Adrenal gland, neonate (10 days old) with echovirus. Diffuse necrosis with hemorrhage. (Courtesy of Dr. A. King, Cambridge, England.) (H&E, ×50)

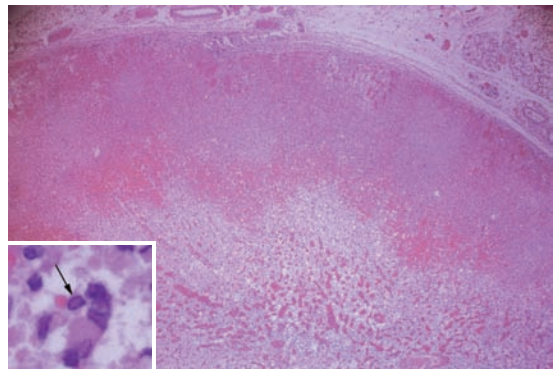


Figure 24.22. Adrenal gland, neonate (12 days old) with herpes simplex virus type 1. Punctate cortical necrosis with surrounding hemorrhage and hyperemia, visible to the naked eye. (H&E, ×50) Inset: Intranuclear viral inclusions (arrow) (H&E, ×400).

changes. Diffuse necrosis with hemorrhage is typical of echovirus infection (Fig. 24.21), while in neonatal herpes there is multifocal, punctate cortical necrosis with surrounding hyperemia or hemorrhage (Fig. 24.22).

Tumors of the Adrenal Gland

Congenital Neuroblastoma

Neuroblastoma is the most common congenital malignant tumor (Fig. 24.23); see also Chapter 15). The tumor is composed of small cells with dark ovoid nuclei. The cytoplasm, which is scanty, has a fibrillary nature and the cells may form rosettes.

Adrenal Cortical Tumors

These are rare in childhood and show incidence peaks in both infancy and adolescence. There is a marked female preponderance (female-to-male ratio of 2.5:1 to 5:1) and an association with the Beckwith-Wiedemann syndrome and hemihypertrophy. The majority of tumors are active, Cushing's syndrome and aldosteronism being common in benign tumors, and virilization (or feminization) being more frequent in carcinomas. The histological criteria used to predict malignant behavior in adults are not reliable in children. Cagle et al. (1986) found that severe nuclear pleomorphism, mitotic activity, necrosis, fibrotic bands, capsular invasion, and even invasion of smaller vessels did not necessarily predict malignancy in children. The only reliable predictor of behavior was tumor size. All tumors <100 g were benign, and all tumors >500 g were malignant.

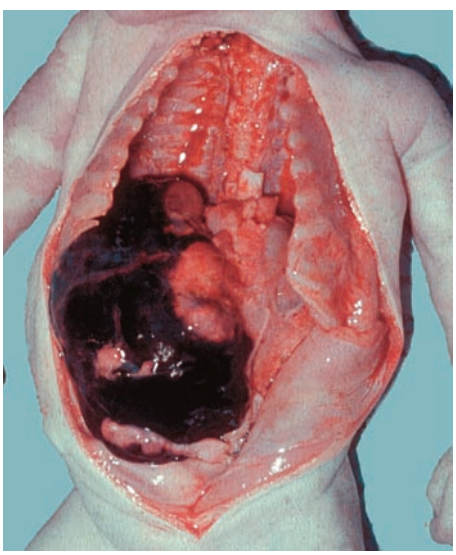


Figure 24.23. Term neonate with massive right-sided congenital neuroblastoma.

Thyroid Gland

Ontogeny

The thyroid develops between the 2nd and 7th weeks from an outpouching in the floor of the mouth. This median portion travels caudally into the neck and is joined by contributions from the fourth pharyngeal pouch. By the 7th week, the buccal connection with the median portion is severed. Between 8 and 11 weeks' gestation there occurs differentiation with follicle formation, concentration of iodine, and formation of thyroxine. From this stage, the fetal thyroid is vulnerable to damage by radioactive iodine. Although the different components of the hypothalamic/pituitary/thyroid axis are functioning by the 12th week, it is between 12 and 18 weeks that full regulatory interactions are established. After 20 weeks' gestation, T_4 levels rise and TSH levels fall, indicating an effective feedback mechanism (Hobel 1980). These changes in hormone levels are reflected in the evolution of the histological appearances of the fetal thyroid (Bocian-Sobkowska et al. 1997).

Maternal T_4 crosses the placenta, but only in very small amounts unless maternal levels are abnormally high, for example, in thyrotoxicosis. Maternal T_4 normally accounts for 30% of the fetal T_4 level. Long-acting thyroid stimulator (LATS), an immunoglobulin G (IgG), also crosses the placenta and stimulates the fetal thyroid, producing fetal thyrotoxicosis and goiter.

In utero, fetal T_4 is converted to reverse triiodothyronine (rT_3), not the active form T_3 (Hobel 1980). Just before birth a switch to T_3 production

occurs to facilitate extrauterine survival. The mechanism is unclear, but there is a surge of fetal TSH at birth followed by an increase in T_3 and T_4 (Fisher and Odell 1969). A more chronic hypersecretion of TSH occurs in the first 24 to 48 hours, which can be significantly increased by cooling the infant. Perhaps this increased thyroid activity effects the histological changes found in the neonatal thyroid (see below).

The parafollicular (C cells) of the thyroid develop from the ultimobranchial body and secrete calcitonin, which inhibits osteoclasts and stops dissolution of bone. They express antigens in fetal life, which supports the hypothesis that they are the origin of mucoepidermoid thyroid cancer (Harach et al. 1993).

Histological Variation in Neonatal Thyroid

Two histological patterns are seen in the neonatal thyroid (Fig. 24.24). The more common is of small uniform follicles lined by cuboidal epithelium, containing a variable amount of colloid. The stroma is more abundant than in the adult thyroid. In the other, the centers of acini are filled by masses of cells. The second pattern was considered a postmortem artifact (Potter and Craig 1976). However, Sclare (1956), Sagreiya and Emery (1970), and Larroche (1977) consider it physiological and labor-related, occurring in normal infants between the 2nd and 7th days. Our present, albeit incomplete, knowledge of the transition from intrauterine to neonatal thyroid function would support this pattern as functional rather than artifactual. In normal human neonates, born vaginally, there is a sharp rise in T_3 and T_4 levels 24 to 48 hours postpartum, and TSH levels rise sharply at birth to very high levels (De Zegher et al. 1994).

Pathology

Malformations

Thyroid agenesis and hypoplasia cause congenital hypothyroidism. In the ectopic thyroid gland, thyroid tissue may be found at any point along the track followed by the thyroid in its migration from the posterior area of the tongue. Thyroid tissue at the base of the tongue is called a lingual thyroid. Occasionally in thyroid gland agenesis the lingual thyroid is the only thyroid tissue

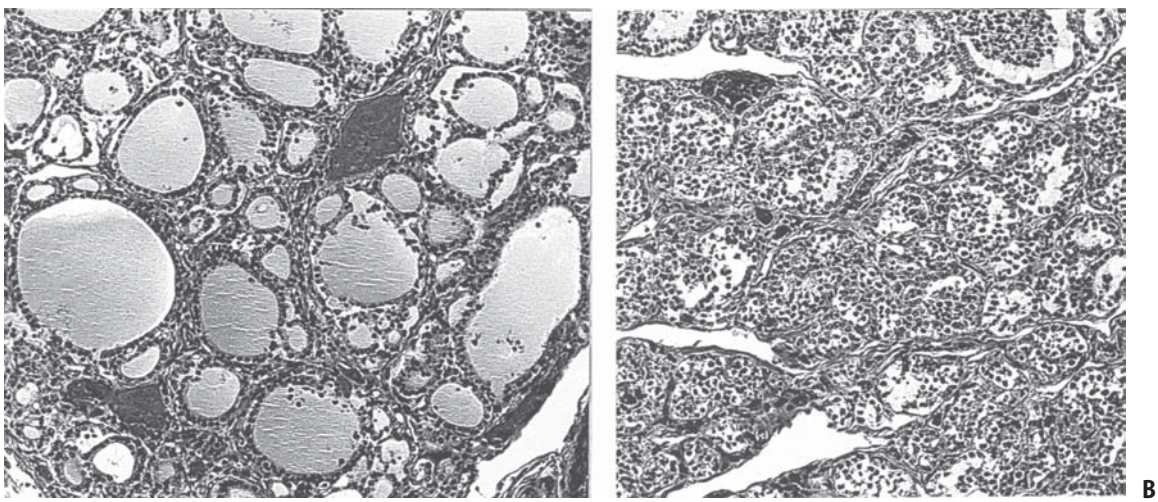


Figure 24.24. Neonatal thyroid showing different histological patterns. (A) Follicles filled with colloid. (B) Follicles filled with desquamated cells with pyknotic nuclei. (A,B: H&E, ×160)

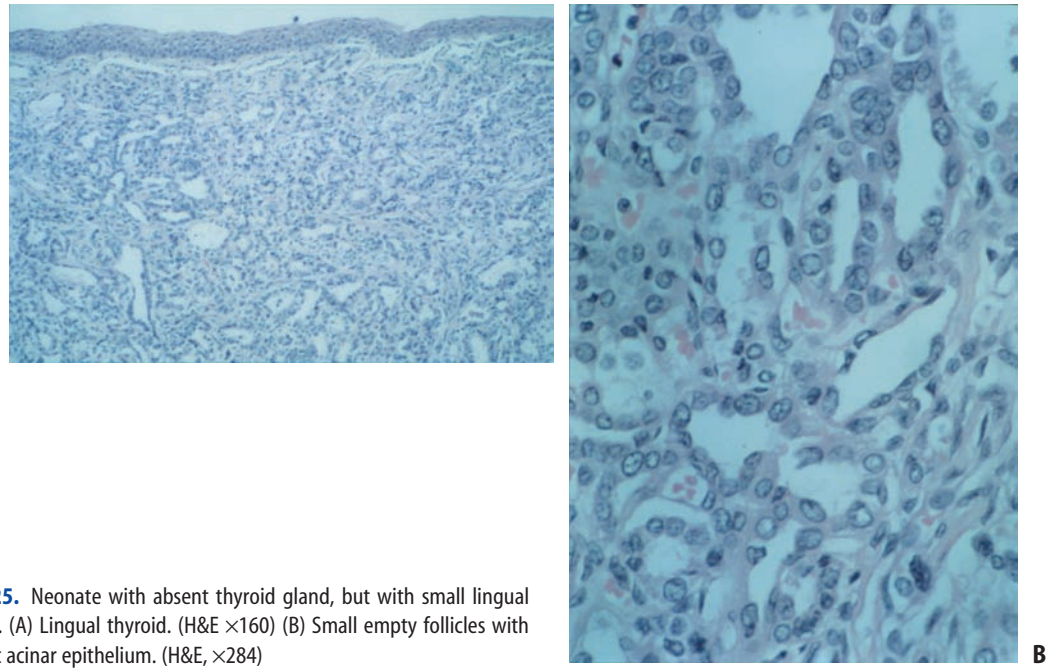


Figure 24.25. Neonate with absent thyroid gland, but with small lingual thyroid rest. (A) Lingual thyroid. (H&E ×160) (B) Small empty follicles with hyperplastic acinar epithelium. (H&E, ×284)

present. In all cases of thyroid agenesis, careful sampling by multiple blocks of the posterior tongue is necessary to identify these ectopic thyroid acini (Fig. 24.25A). In thyroid agenesis, these foci show compensatory hyperactivity (Fig. 24.25B). Thyroid tissue has been described at various sites within the mediastinum.

Congenital Hypothyroidism

Congenital hypothyroidism may be associated with mental retardation and neurological defects including ataxia, spasticity, strabismus, and poor muscle tone (i.e., cretinism). Not all hypothyroid neonates have this neuronal damage because sufficient levels of maternal thyroxine have crossed the placenta and protected the fetal brain (Pemberton et al. 2005). When fetal brain damage has occurred, it cannot be reversed by neonatal thyroxine treatment (Fisher 1997). Fortunately, most cases of fetal hypothyroidism are sporadic and occur in euthyroid mothers in whom maternal T_4 can protect the fetal CNS. Normal postnatal growth and development will occur in this group of hypothyroid infants if they are adequately treated after birth.

Primary Congenital Hypothyroidism

There are three main causes of primary hypothyroidism in neonates: thyroid dysgenesis (1 in 5000 births), which may be due to agenesis or hypoplasia; a defect in hypothalamic/pituitary function (1 in 20,000 births); and familial goiter or thyroid dyshormonogenesis (1 in 30,000 births). This last condition, if associated with deafness, is called Pendred's syndrome.

Dyshormonogenesis rarely presents in the neonatal period, being more usual at puberty. Some cases have presented in utero with polyhydramnios (Perelman et al. 1990) and been successfully treated with intraamniotic T_4. Dyshormonogenesis is due to a defect in thyroid hormone biosynthesis secondary to a deficiency of one of the enzymes of which peroxidase, the enzyme responsible for the organic binding of iodide, is the most commonly involved. The enzyme defects are inherited in an autosomal-recessive fashion.

Secondary Congenital Hypothyroidism

The thyroid may be severely damaged in utero, either by toxic chemicals crossing the placenta (i.e., the antithyroid drugs thiouracil or methimazole) or radioactive iodine given after the 10th week of gestation. Such affected fetuses should be treated by intraamniotic thyroxine. Topical or ingested iodine used during pregnancy can cause transient congenital hypothyroidism (Danziger et al. 1987).

Worldwide, secondary hypothyroidism is most commonly caused by dietary lack of iodine or endemic goitrous cretinism. These infants are born in areas where adult goiter is endemic, but only those infants whose mothers have very low levels of thyroxine will display the features of cretinism.

The appearance and clinical findings of fetal hypothyroidism and neonatal cretinism are well known. In brief, the affected infant may be small for dates, fails to thrive, and frequently suffers from prolonged physiological jaundice and constipation. Radiographs show delay in ossification centers with small and fragmented femoral epiphyses. The skin is dry, the hair is coarse, and the tongue protuberant (Fig. 24.26).

Not all cases of hypothyroidism have this easily recognizable phenotype. The TSH levels are raised in all cretins except those resulting from failure of fetal hypothalamic–pituitary function. T_4 levels are always low. It is a relatively common disorder [1 in 4000 births; cf. phenylketonuria (PKU) at 1 in 20,000], which, if not treated early, can cause irreversible brain damage. There is a strong argument for mass neonatal screening.

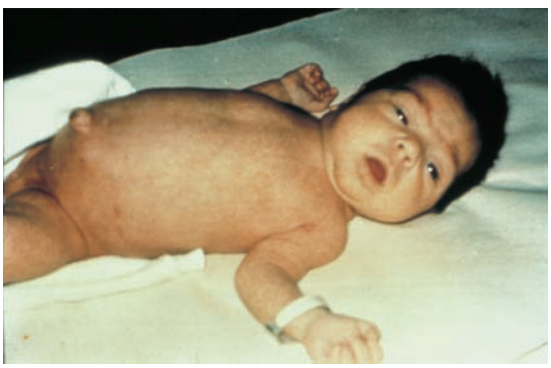


Figure 24.26. Hypothyroid neonate. Coarse hair and facies; umbilical hernia.

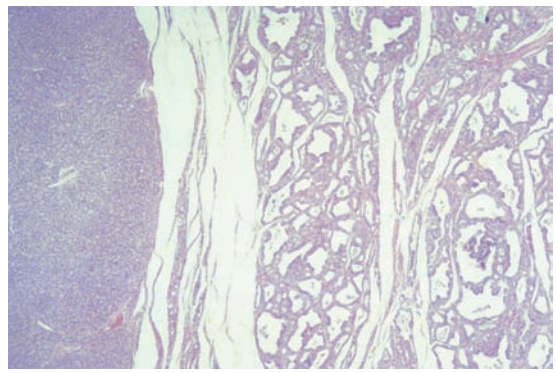


Figure 24.27. Dyshormonogenetic thyroid gland. Hyperplastic nodule. Adjacent thyroid shows hyperplasia and papillary infolding. (H&E, ×140)

The size of the thyroid gland in congenital hypothyroidism varies. In thyroid dysgenesis, irradiation damage, and in a minority of endemic cases, the thyroid is absent or very atrophic. In those dyshormonogenetic cases that present at birth, the thyroid is usually enlarged and nodular. Histological examination reveals nodular hyperplasia with small empty follicles lined by cells with focal nuclear pleomorphism and gigantism (Figs. 24.27 and 24.28). This appearance has been mistaken for thyroid carcinoma.

The majority of endemic cases have enlarged thyroids weighing 5 to 10 g (normal, 1 to 3 g). Follicles are large and the amount of colloid increased. Some infants in areas of endemic goiter have large goiters but are euthyroid. Their large goiters have small follicles and hyperplasia of acinar cells.

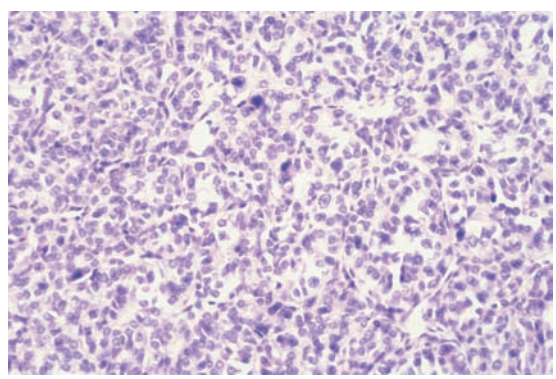


Figure 24.28. Dyshormonogenetic thyroid gland, hyperplastic nodule. There is nuclear pleomorphism and small empty follicles. (H&E, ×284)

Congenital Thyrotoxicosis

Occasionally, thyrotoxic infants are born to mothers with thyrotoxicosis. As maternal TSH does not cross the placental barrier, the explanation is that maternal thyroid-stimulating IgG is responsible. Histological examination shows a hyperplastic epithelium filling the acini and colloid depletion. Affected infants are irritable, and have diarrhea and thyrotoxic cardiac failure. Twenty percent die in the neonatal period if not rendered euthyroid by carbimazole given during pregnancy (Heckel et al. 1997) or to the neonate.

Neonatal Goiters in Euthyroid Infants

Enlarged thyroids in euthyroid infants are a feature of thyroid dyshormonogenesis, some cases of environmental iodine deficiency (see above), and certain iatrogenic disorders. Congenital iodide goiter is associated with prolonged use of iodides by the mother, as an expectorant in asthma and chronic bronchitis. Iodide crosses the placenta and inhibits peroxidase activity. Fetal T_4 levels fall and TSH levels increase. This causes hyperplasia of the gland, epithelial hyperplasia, and varying degrees of colloid storage (Iancu et al. 1974). The gland may be so much enlarged that it causes respiratory obstruction in the neonate. Para-amino salicyclic acid for maternal tuberculosis may produce a similar type of congenital goiter.

Tumors of the Thyroid

Teratomas are the most common neonatal thyroid tumor and may present with polyhydramnios (see Chapter 15). Congenital carcinoma of the thyroid is extremely rare, and some of the cases reported are possibly examples of thyroid dyshormonogenesis. Occult papillary carcinoma of the thyroid has been reported in a neonate (Mills and Allen 1986).

Parathyroid Glands

Ontogeny

The parathyroids are variable in number and position, but usually number four. The superior pair is derived from the fourth pharyngeal pouch and lies close to the cricothyroid junction. The inferior pair, from the third pharyngeal pouch, is more variable in position but is usually posterolateral to the lower poles of the thyroid, although it may be found in the mediastinum within the thymus. Functionally there is only one cell type, which assumes three histological forms: chief cell (basic cell type), oxyphil cell, and water clear cell. Only chief cells are found in the fetus and neonate. They secrete parathormone (PTH), which is controlled by the concentration of calcium ions in fluid perfusing the glands. Functional PTH can be detected in human fetal parathyroid from the 12th week, but blood levels are very low or undetectable because of suppression by the hypercalcemic status of the normal fetus (Fleischman 1980).

Hypercalcemia is achieved by an active placental pump of calcium from mother to fetus against a positive gradient; by this means the fetus accrues 25 to 30 g of calcium by term. Calcitonin, secreted by parafollicular cells in the thyroid, inhibits bone dissolution, and its level in fetal serum is elevated above the adult normal level.

The combination of high calcitonin and hypercalcemia enhances bone accretion and mineralization of the fetal skeleton. Neither PTH nor calcitonin crosses the placenta, unlike the third hormone important in calcium homeostasis, 1,25-dihydroxycalciferol vitamin D. Infants born with low levels of vitamin D, usually because of low maternal levels, have an increased incidence of early neonatal hypocalcemia.

Pathology

Ectopia of the parathyroids, especially the inferior group, is common; the thyroid, thymus, and mediastinum are the usual sites (Harach and Vujanic 1993). Ectopic parathyroid tissue has also been reported in the pericardium and in the wall of a thymic duct cyst.

Fetal and Neonatal Hypoparathyroidism

As PTH seems unnecessary for fetal calcium homeostasis and bone formation, deficient infants appear normal at birth. However, when falling blood calcium levels in the early neonatal period fail to trigger PTH secretion, severe hypocalcemia develops with tetany and convulsions. There is hyperphosphatemia.

Primary Hypoparathyroidism

This condition accompanies aplasia or hypoplasia of the parathyroid tissue in malformations involving the 3rd and 4th branchial clefts, for example, DiGeorge syndrome, where there is associated thymic aplasia and malformation of the heart and aortic arch.

Idiopathic Hypoparathyroidism

This condition rarely presents in the neonatal period. Some cases appear to have an X-linked recessive inheritance pattern (Peden 1960).

Secondary Hypoparathyroidism

This condition occurs in babies of women with severe hypercalcemia in pregnancy, usually complicating sarcoidosis, excessive vitamin D intake, or hyperparathyroidism. Whatever the cause, maternal hypercalcemia increases transport of calcium across the placenta, so that severe chronic fetal hypercalcemia develops. This totally suppresses, and may even permanently damage, the infant's parathyroid function, so that neonatal hypocalcemia develops.

Pseudohypoparathyroidism and Pseudopseudohypoparathyroidism

The pseudo forms of hypoparathyroidism present in later childhood and are not due to parathyroid disorder.

Fetal and Neonatal Hyperparathyroidism

Hyperparathyroidism produces hypercalcemia, which, in the neonate, presents with hypotonia, dehydration, and respiratory distress. Generalized demineralization of the skeleton, subperiosteal resorption, osteitis fibrosa cystica, and even spontaneous rib fractures occur in severe untreated cases. The condition may be primary or secondary.

The only sure way to distinguish the primary and secondary fetal forms is by detailed study of the mother's own calcium status. This is very important for genetic counseling and management of future pregnancies.

Primary Familial Hyperparathyroidism

Two main familial forms have been described: an autosomal recessive type, neonatal severe hyperparathyroidism (NSPH), and an autosomal dominant form, which is usually part of the syndromes of multiple endocrine neoplasia (MEN), especially MEN-2; MEN presents in older children. In the cases of NSPH presented by Nishiyama et al. (1990), there was severe nephrocalcinosis and renal tubular acidosis.

Secondary Fetal Hyperparathyroidism

This condition, also known as neonatal rickets, occurs in infants as a response to chronic intrauterine hypocalcemia. Fetal hypocalcemia is due to diminished calcium transport across the placenta and is secondary to severe maternal hypocalcemia. This may be caused by maternal hypoparathyroidism, severe dietary deficiency of vitamin D (as in Asian immigrants in the United Kingdom) and chronic renal failure.

Secondary Neonatal Hyperparathyroidism

Premature infants and neonates with malabsorption caused by biliary atresia may develop secondary hyperparathyroidism. The glands are only slightly enlarged and show uniform hyperplasia of the chief cells. Occasionally, nodular hyperplasia develops.

Early Neonatal Hypocalcemia

Early neonatal hypocalcemia occurs in approximately one third of premature, sick neonates and is probably caused by failure of calcium homeostasis as a result of low vitamin D levels. Although the premature infant can eventually achieve adequate PTH levels, and has increased calcitonin levels, it frequently has decreased levels of 1,25-dihydroxycalciferol vitamin D. This arrangement possibly tips the balance toward retaining calcium in the bone and diminishing the uptake from the gastrointestinal tract, thus causing hypocalcemia.

Early neonatal hypocalcemia is more common after perinatal asphyxia or respiratory distress syndrome. It is a particular hazard for infants of diabetic mothers. The reason for this association is not clear, but probably relates to the lower levels

of 1,25-dihydroxycalciferol present in the serum of diabetic mothers at term. Hypocalcemia is also found in hypomagnesia and hypoparathyroidism (see above).

Endocrine Pancreas

Ontogeny

The pancreas arises from two diverticula from the dorsal and ventral surfaces of the gut. As well as producing digestive enzymes (exocrine pancreas), the pancreas contains cells, mainly within the islets of Langerhans (endocrine pancreas), that produce a group of polypeptide hormones and are part of the amine precursor uptake and decarboxylation (APUD) system. These peptide hormones are concerned with regulating the utilization and storage of nutrients once they reach the bloodstream. There are four cell types in the endocrine pancreas: A cells produce glucagon, B cells produce insulin, D cells produce somatostatin, and PP cells probably produce a variety of polypeptide hormones. G cells, which produce gastrin, may also occur in the pancreas.

The origin of these endocrine cells is controversial (see Introduction p. 662). The A cells appear as early as 9 weeks and the B cells at 10 to 11 weeks. They appear to develop in two generations (Liu and Potter 1962). The first generation comes from paratubular cell buds, later detaching and establishing the primary islets, which continue to enlarge until 20 weeks' gestation, after which they disintegrate. From about 16 weeks the second generation of islets arises from terminal ducts and forms secondary islets, which continue to increase in number and size to become typical adult islets. Because of this pattern of development the fetal and neonatal endocrine pancreas is much more diffuse and visibly occupies a higher proportion of the pancreatic volume than in older children and adults. It is important to remember this difference when assessing a neonatal pancreas for nesidioblastosis (excess endocrine tissue).

Insulin in B cells is detectable as early as 10 weeks, and prolonged hyperglycemia elevates fetal insulin levels (Obenshain et al. 1970), although less rapidly than certain amino acids (Milner et al. 1971). Glucagon in A cells appears at 8 to 9 weeks. In utero, glucagon shows no response to low glucose levels, adopting its regulatory role only after birth. However, glucagon levels are raised in fetal and perinatal distress (Hill 1980) and in erythroblastosis fetalis.

Histological study of the endocrine pancreas is more satisfactory if the tissue has been fixed in Bouin's solution. Although traditional stains can be used to differentiate the cell types, this is done more reliably by immunocytochemical techniques.

Normal Histological Variations in the Fetal and Neonatal Pancreas

In the fetal and neonatal pancreas, there is a variable but higher proportion of non-islet endocrine cells than in the adult (Fig. 24.29), and the percentage of pancreas occupied by endocrine tissue in the neonate (10%) (Jaffe et al. 1980) is much higher than in the adult (1% to 2%). This diffuse involvement might suggest a pathological excess of endocrine tissue (diffuse nesidiodysplasia, see Persistent Hyperinsulinemic Hypoglycemia of Infancy: Nesidioblastosis p. 687), so it is most important in

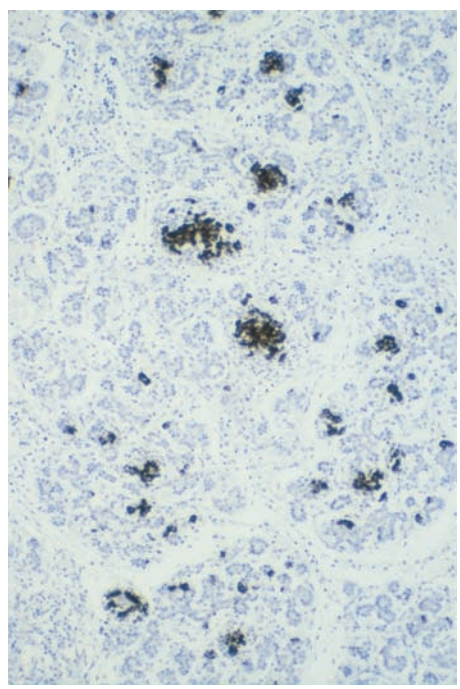


Figure 24.29. Fetal pancreas, 14 weeks' gestation. Diffuse pattern of endocrine cells. Stained for insulin. (PAP, ×145)

such cases to compare with several age-matched controls. There is also proportionately more endocrine tissue in the body and tail of the pancreas than in the head, so that sampling should also be comparable. Minor cellular infiltrations are relatively common. The infiltrate is usually of the erythromyeloid series and most marked in preterm infants. If myeloid, it may indicate a generalized leukemoid reaction, as occurs in infection and trisomies 21 and 18. Occasionally, the infiltrate is predominantly lymphocytic. Liu and Potter (1962) found 50% of fetuses had a lymphocytic infiltrate, which was most marked between the 6th and 8th months, but present also at term. It has been postulated that this infiltrate is related to the natural degeneration of the primary islet tissue and of A cells in particular.

Pathology

Absence

Absence of the endocrine pancreas occurs in total pancreatic agenesis. Maternal insulin does not cross the placenta; thus these infants have total insulin deficiency. They are severely growth restricted, achieving only 28- to 30-week fetal size at term, lack fat deposition, and have poor muscle development. Staffers et al. (1997) identified homozygosity for a single nucleotide deletion in the human *IPF1* gene coding sequence, the *IPF1* gene being important in pancreatic development. Pancreatic agenesis is very rare and possibly has an autosomal recessive inheritance.

Hyperplasia

The conditions in the fetus and neonate in which there is hyperplasia of the endocrine pancreas are listed in Table 24.1.

Table 24.1. Conditions associated with hyperplasia of the endocrine pancreas

Infants of diabetic mothers (IDM)
Erythroblastosis fetalis
Beckwith–Wiedemann syndrome
Zellweger's syndrome (occasionally)
Neonatal hepatitis (occasionally)
Intrauterine growth restriction (occasionally)
Persistent hyperinsulinemic hypoglycemia of infancy
Diffuse nesidioblastosis
Focal nesidioblastosis
Adenoma

Infants of Diabetic Mothers

Infants of diabetic mothers (IDMs) have many problems; the most frequent are increased somatic size (macrosomia) (Fig. 24.30), increased incidence of perinatal death, increased frequency of malformation, and hypertrophy of the islets of Langerhans, with B-cell hyperplasia and hyperinsulinemia. Morriss (1984), in an excellent review, explains many of these features, and many other clinical problems experienced by the IDM in the neonatal period, as being the result of persistent maternal hyperglycemia. However, maternal hyperglycemia cannot be the only cause of islet hyperplasia, as both it and fetal hyperinsulinism have been found in IDMs as early as 4 months' gestation, and the B cell is not responsive to glucose levels until after 24 weeks (Bloodworth 1982). Interestingly, ionic stimuli, leucine, and arginine (Milner et al. 1971) are known to stimulate fetal B cells from 14 weeks onward, and maternal hyperaminoacidemia has been reported even in the mildest gestational diabetes (Milner 1979). This may explain the islet hyperplasia in some infants of prediabetic women (Fig. 24.31). The fetal hypothalamic-pituitary axis has an ill-understood role in B-cell function: anencephalic infants of diabetic mothers fail to develop B cell hyperplasia (Van Assche et al. 1969).

Histological examination of the pancreas in IDM shows an increased islet cell mass of about three times normal (Fig. 24.31A). This is due to hypertrophy of the islets (macronesia), neoforma-

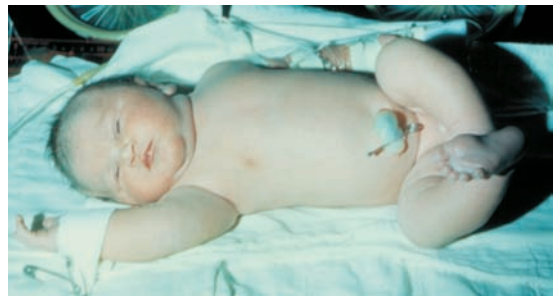


Figure 24.30. Infant of diabetic mother. There is macrosomia and obesity.

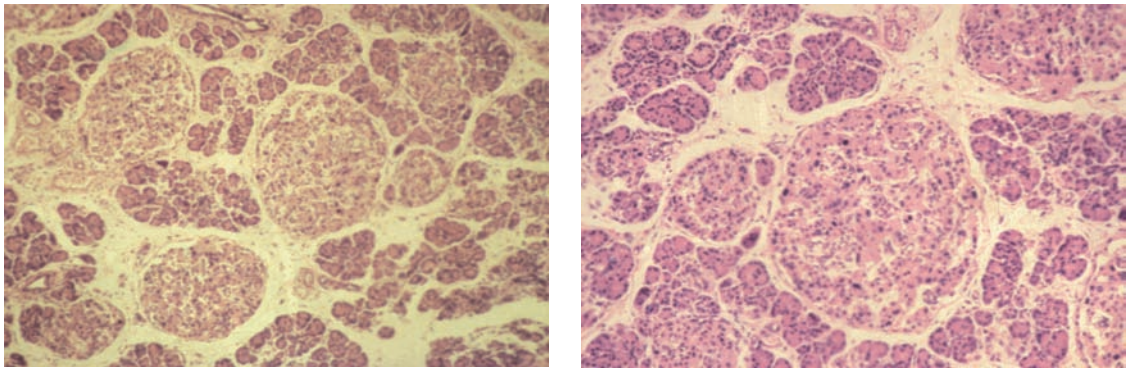


Figure 24.31. Pancreas of neonate of diabetic mother. (A) Increased islet size, no cellular infiltrate. (H&E, ×50) (B) Nuclear pleomorphism and gigantism. (H&E, ×200)

tion of islets (polynesia), and hyperplasia of islet cells. The bulk of the increase is due to a great increase in B cells, but non-B cells are also increased. The B cells usually show nuclear pleomorphism and gigantism (Fig. 24.31B). In contrast the enlarged islets of erythroblastosis fetalis do not show this selective increase of B cells.

Numerous necropsy studies have shown that 34% to 65% of IDMs have cellular infiltration around, but not usually within, some of the hypertrophied islets (Fig. 24.32). Lymphocytes, histiocytes (occasionally with Charcot–Leyden crystals) and neutrophils are also present, but it is the eosinophilic infiltration which is specific to IDM (Fig. 24.33). Eosinophil insulitis is not found in erythroblastosis fetalis, the other condition where islet hypertrophy is associated with cellular infiltration (see Fig. 24.25). It may, however, be seen in the pancreas in infants of prediabetic women (Fig. 24.34). Its presence and significance should be drawn to the attention of the obstetrician. Because eosinophilic infiltrates are not found in other viscera in IDM, its apparently organ-specific nature has caused speculation about a local immune reaction with maternal antiinsulin antibodies. There is considerable experimental and some clinical evidence to support this hypothesis, but the eosinophilic infiltrate has been found in IDMs whose mothers have not had insulin (Silverman 1963) and moreover were considered to be nondiabetic at that time. This does not exclude the possibility of maternal IgG autoantibodies to insulin or islets crossing the placenta to localize in fetal islets. The eosinophil infiltration disappears a few days after birth.

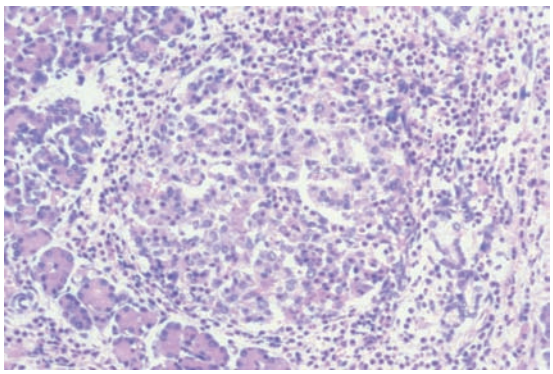


Figure 24.32. Pancreas of neonate (birth weight, 5.2 kg); maternal glycosuria antenatally. Hypertrophied islets, nuclear gigantism, marked periinsular cellular infiltrate. (Courtesy of Dr. A.M. Gibson, Glasgow, Scotland.) (H&E, ×236)

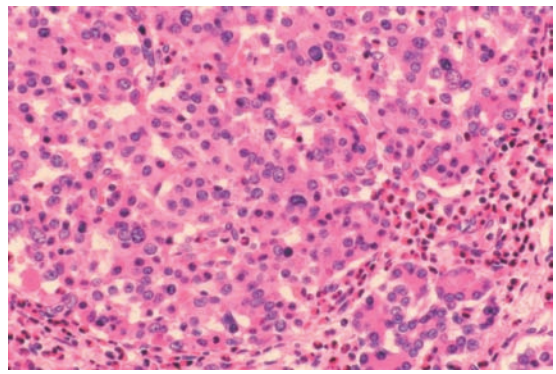


Figure 24.33. Pancreas, same neonate as in Figure 24.32. Cellular infiltrate is eosinophilic. (Courtesy of Dr. A.M. Gibson, Glasgow, Scotland.) (H&E, ×1250)

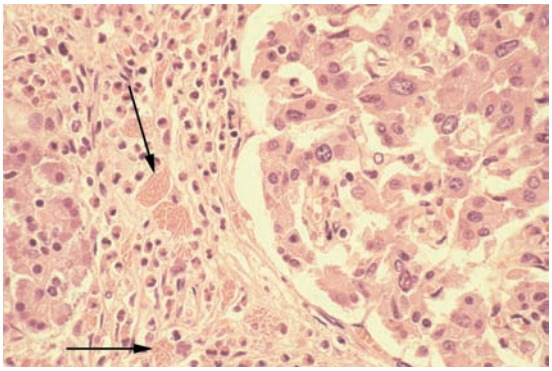


Figure 24.34. Pancreas, neonate of prediabetic mother. Numerous eosinophils and Charcot-Leyden crystals (arrows). (H&E, ×292)

From about 11 days onward there may be fibrosis of the islets, affecting up to 20% of the islet mass (Nelson et al. 1977). Fibrosis may be the sequel to cellular infiltration; it is not usually found in non-IDM infants, does not affect all islets, and may occur in IDMs whose mothers do not receive insulin. These pancreatic changes are independent of the severity of the maternal diabetes and may be seen in infants whose mothers only exhibit diabetes many years later.

Hyperplasia of fetal B cells is associated with fetal hyperinsulinemia, which may lead to neonatal hypoglycemia.

Increased Perinatal Mortality

A feature of the diabetic pregnancy is increased perinatal mortality (PNM). Before 1921 most babies and many diabetic mothers died in pregnancy. Rigorous control of maternal diabetes has reduced overall perinatal mortality to less than 2% (Somville 1990), but this is still two to three times higher than in nondiabetic pregnancies. The risk of fetal loss depends on the duration and severity of maternal diabetes and on the degree of uterine vascular damage. White (1965) defined five classes (A to E) of severity of maternal diabetes and showed a direct correlation between perinatal mortality and the severity of the maternal diabetes. The causes of perinatal and neonatal death in IDM are shown in Tables 24.2 and 24.3, respectively. The increase in PNM was due to an increase in both intrauterine and neonatal death. Despite the many advances in the 40 years since White's depressing figures, there is still a higher rate of fetal malformation, of early and late fetal death, and of infant mortality in these babies. In 1997 the results of a 5-year survey of 462 pregnancies in mothers with preexisting insulin-dependent diabetes was published (Casson et al. 1997). This study, carried out in the U.K., showed that the infants of diabetic women were at both an increased risk of congenital malformation (×10) and of being stillborn (×5), when compared with the general population of pregnant women. The increased risk of fetal death begins in the third trimester and increases gradually from the 30th to 40th weeks. Necropsy of stillborn infants usually fails to reveal a cause of death, although some infants have malformations.

Experimental studies (Milley et al. 1981) have shown that fetal hyperglycemia in lambs causes hypoxemia, increased cardiac output, increased basal metabolic rate (BMR), and diminished cord blood flow. A similar pathophysiological state

Table 24.2. The causes, at necropsy, of perinatal death in 50 IDM (Warren et al. 1966)

Unexplained[a]	36%
Prematurity[b]	26%
Hyaline membrane disease	16%
Birth injury (dural fold tears)	8%
Pneumonia	6%
Malformations	6%
Adrenal hemorrhage	2%

[a]Includes 11 macerated stillbirths.
[b]Defined as <32 weeks or <1500 g.

Table 24.3. Primary causes of neonatal death in 39 infants of diabetic mothers (IDMs) compared with 70 non-IDMs (Hubbell et al. 1965)

	IDM[a] (≥2500 g)		Non-IDM	
	No.	%	No.	%
Extreme prematurity	1	2.5	7	10
Hyaline membrane disease (HMD)	18	46	23	33
Respiratory pathology (not HMD)	3	8	18	26
Congenital malformations	10	26	5	7
Infection (nonpulmonary)	6	15	1	1
Neurological (asphyxial)	0	0	10	14
Other	1	2.5	6	9
Total	39	100	70	100

[a]All deaths occurred to babies ≥37 weeks' gestation.

in humans may be the cause of unexplained intrauterine deaths in diabetic pregnancies. The stillbirth rate has been reduced dramatically by strict control of maternal hyperglycemia.

Macrosomia

This condition is present in many IDMs and consists of enlarged viscera, particularly liver, heart, and adrenals, and adiposity. The brain is often smaller than normal. The thymus may show cortical involution.

The cause of macrosomia and adiposity is partly the combination of fetal hyperglycemia and fetal hyperinsulinemia (insulin does not cross the placenta), which results in the conversion of glucose to fat and protein. Macrosomia does not appear before 25 weeks' gestation, possibly because of low fetal tissue sensitivity to insulin action before this. Not all IDMs are macrosomic and, if there is severe maternal vascular disease diminishing blood flow to the placenta, the fetus may show intrauterine growth restriction.

Macrosomia greatly increases the risk of shoulder dystocia and cephalopelvic disproportion, producing birth injury and intrapartum asphyxia. Although the incidence of macrosomia is reduced by good control of the diabetes, the condition can precede the onset of maternal diabetes, suggesting a more complex etiology than merely fetal and maternal hyperglycemia.

Cardiomegaly

Even in the absence of macrosomia, cardiomegaly occurs in IDMs and 30% have transient hypertrophic cardiomegaly (Gutgesell et al. 1980). There may be generalized ventricular hypertrophy with asymmetric hypertrophy of the septum. If very severe, it may induce cardiac failure as the result of obstruction of the outflow (hypertrophic obstructive cardiomyopathy, HOCM). Histologically, there is whorling and disorganization of the muscle fibers in the septum and there may be foci of necrosis (Gutgesell et al. 1980), constituting cardiomyopathy.

The cause is not known but could be due to increased cardiac outflow in IDMs or to direct action of insulin on fetal myocardium, which is very rich in insulin receptors.

Congenital Malformations

There is an increased risk of most congenital malformations in diabetic pregnancies. In a large retrospective study (Janssen et al. 1996) carried out in Washington state between 1984 and 1991 of mothers with established diabetes, gestational diabetes, and nondiabetic controls, it was shown that the prevalence of congenital malformations in each group was 7.2%, 2.8%, and 2.1%, respectively. This is a lower increase in risk (×3) than that (×10) shown in a smaller study (Casson et al. 1997) from the U.K. An earlier large prospective study has shown that infants of insulin-dependent diabetics are eight times more likely to have a major malformation (Becerra et al. 1990). Contrary to some earlier reports, there is also an increase in minor malformations (Rosenn et al. 1990). An increase in anencephaly, spina bifida, hydrocephaly, coloboma, transposition of great vessels, ventricular septal defect, patent ductus arteriosus, and limb flexion contractures is found (Becerra et al. 1990). Neave (1984) found an increased frequency of single umbilical artery and of microcephaly. Sacral dysgenesis and other caudal regression syndromes have been linked with IDM, but Becerra et al. (1990) found only two of nine cases of caudal regression had a history of maternal diabetes. A large study of limb reduction defects in Sweden between 1973 and 1986 showed no association with maternal diabetes (Kallen 1989). More subtle neurobehavioral damage has also been recorded in long-term follow-up studies of IDMs (Rizzo et al. 1997). Interestingly, the large United States study (Janssen et al. 1996) showed that female infants of diabetic mothers were twice as likely to be malformed.

Congenital malformations are more frequent in infants of women with poorly controlled diabetes (Miller et al. 1981). Studies of glycosylated hemoglobin, an index of long-term glucose control, have shown that elevated levels in the first trimester increase the risk of congenital malformations (Reece and Hobbins 1986; Rosenn et al. 1990). Malformation rates were related to the severity of diabetes using White's classification. However, Damm and Molsted-Pedersen (1989) have shown a fourfold decline in major malformations in White classes D and E between 1977 and 1986, with loss of the previously observed correlation

between the severity of the maternal diabetes and the frequency of malformations. This probably reflects better diabetic control, although adequate insulin control experimentally (Wentzel and Eriksson 1996) and clinically does not completely remove the increase in malformations. The exact etiology of diabetic fetopathy is not clear but presumably relates to the altered metabolic milieu at conception and during embryogenesis. Altered levels of scavenging enzymes have been found in rat embryos exposed to a diabetic type environment, suggesting that free radicals may be important (Forsberg et al. 1996).

Moley et al. (1998) showed that preimplantation mouse blastocysts exposed to hyperglycemic conditions develop premature and increased programmed cell death (apoptosis), which is due to an increased expression of Bax, a member of the Bcl-2 protein family. Moreover, this inappropriate apoptosis was abolished by insulin treatment of the maternal diabetes before and after conception.

Placental Pathology

The placenta in IDM tends to be heavy, but if there is maternal vascular disease the placenta is small and infarcts are present (see Chapter 3). There is an increased incidence of single umbilical artery (3% to 5%). Placental pathology in IDM is discussed by Fox (1997).

Other Complications

Polyhydramnios is common in IDMs. There is an increased susceptibility in late gestation and in the neonatal period to spontaneous thrombosis of veins (e.g., renal vein, inferior vena cava, umbilical vein) and arteries (e.g., ulnar and femoral). The venous thromboses can be the source of neonatal paradoxical thromboembolism, and the arterial thrombosis can be a cause of intrauterine gangrene. In the neonatal period IDM are susceptible to hypocalcemia (see Early Neonatal Hypocalcemia p. 680), renal vein thrombosis, hyperbilirubinemia, and, most importantly, respiratory distress syndrome. This last condition, which was a major cause of death in IDMs in the past, is possibly due to retarded production of one fetal lung phospholipid important for effective surfactant action (Cunningham et al. 1978).

Infants of Maternal Gestational Diabetics

These infants may show some of the clinical features found in IDMs but are usually less severely affected. Nonetheless, their metabolism is abnormal, producing hyperplasia of the islets of Langerhans and often macrosomia (≥4000 g). There is a significant increase in congenital heart disease in infants of women with gestational diabetes who need insulin during pregnancy. In a small study of gestational diabetes it appeared that very tight control of glucose levels removed the previously recorded increase in perinatal mortality (Thompson et al. 1994). There is a 56-fold risk of developing established diabetes in the pregnancy following a pregnancy complicated by gestational diabetes and a sixfold risk after delivering a macrosomic infant (McGuire et al. 1996).

Buchanan and Kitzmiller (1994) have published an interesting review of diabetic fetopathy.

Beckwith–Wiedemann Syndrome

The Beckwith–Wiedemann syndrome (BWS) is characterized by exomphalos, macroglossia, visceromegaly, mild microcephaly, gigantism, hemihypertrophy, and hyperinsulinemic hypoglycemia. Histological examination shows hyperplasia of many organs, most notably islet cell hypertrophy, without a generalized pancreatic endocrine tissue increase, and adrenal cytomegaly (see Fig. 24.14). There is an increased risk of a wide range of tumors, but particularly nephroblastoma, adrenal cancer, hepatoblastoma, and rhabdomyosarcoma. An excellent review of the clinical, pathological, and molecular genetic aspects of BWS has been published by Cohen (2005).

There has been evidence for some time that this syndrome of fetal overgrowth and tumor development is caused by dysfunction of fetal growth regulating factors (Ashton and Aynsley-Green 1978; Aynsley-Green 1982). Genomic imprinting is a mechanism of gene regulation with imprinting mistakes resulting in genetic disorders. A cluster of imprinted genes, all of which encode for important fetal growth factors, are located together in the 11p15.5 region. These imprinted genes exist in two separate regulatory domains at 11p15. Domain 1 contains two genes—*IGF2* and *H19*, the insulin-controlling gene. Domain 2 includes a range of genes of which three have been

implicated in BWS. Many of the familial and sporadic cases have shown genotypic abnormalities in the 11p15 region, including paternal disomy for 11p15.5, which would explain the overexpression effect. Recent work has suggested that the maternally imprinted gene *Igf2,* which encodes a fetal insulin-like growth factor, is overexpressed in the BWS. This hypothesis has now been successfully tested by introducing the *Igf2* transgene into a mouse genome and so producing embryos with overexpression of *Igf2* (Sun et al. 1997). These mice developed most of the features of BWS, but do not show exomphalos or an increased incidence of tumors. Similarly, overexpression of *H19* has been shown to occur in some cases of BWS (Catchpoole et al. 1997). It appears that the clinical heterogenicity of BWS is partly a reflection of the genotypic heterogenicity. The paternal disomy form of BWS often shows tissue mosaicism and is closely linked to hemihypertrophy, whereas alteration of genes in domain 1, especially of *H19,* is associated with a high risk of malignancy, in particular nephroblastoma (Weksberg et al. 2005). Our lack of understanding of hypermethylation in general and its role in altering gene imprinting in particular, means that we still have much to learn about BWS.

Beckwith-Wiedemann syndrome is more common following in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (Allen and Reardon 2005), apparently because cell culture conditions may adversely affect DNA methylation (Gosden et al. 2003)

Erythroblastosis Fetalis

In severe erythroblastosis fetalis there is hyperplasia of the islets with cytomegaly, and the interstitium is infiltrated by erythropoietic cells (Fig. 24.35). The cause of islet hyperplasia is not known but it is functional, so that neonatal hypoglycemia is a hazard in the newborn. Unlike the islet hyperplasia in IDMs, where B cells predominate, normal distribution of insulin- and glucagon-secreting cells is maintained within the hyperplastic islets.

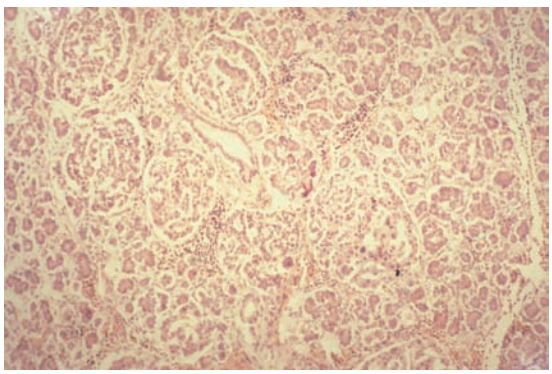
Islet Hyperplasia and Liver Disease

Zellweger's (cerebrohepatorenal) syndrome is characterized by abnormal facies, hypotonia, hepatic fibrosis, renal microcysts, and abnormalities of the CNS. Several reported cases have shown hyperplasia of the islets of Langerhans. The syndrome is autosomal recessive and of uncertain etiology.

Hyperplasia of the islets may be secondary to other liver disease; hyperplasia is found at necropsy in some neonates dying of neonatal hepatitis (Greco and Finegold 1973). The relationship between islet function and liver disease is unknown but could be due to elevated levels of amino acids that are tropic to fetal and neonatal islets (Milner 1979).

Persistent Hyperinsulinemic Hypoglycemia of Infancy: Nesidioblastosis

Many conditions produce transient or short-term hypoglycemia in neonates, including the



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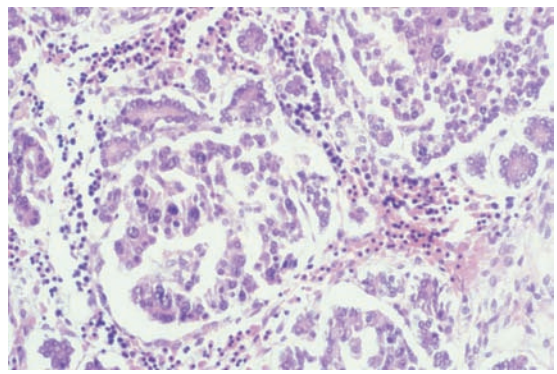


B

Figure 24.35. Pancreas, term neonate: erythroblastosis fetalis (anti-Kell). (A) Hyperplastic islets, nuclear gigantism, interstitial infiltration [could be mistaken for an infants of a diabetic mother (IDM)]. (H&E, ×200) (B) Infiltrate is erythropoietic (normoblasts). Compare with Figure 21.34. (H&E, ×800)

conditions causing islet hyperplasia already mentioned. In 20% to 50% of cases, hypoglycemia is persistent and severe and associated with inappropriately high insulin levels. If untreated, the infant dies; survivors may show permanent hypoglycemic brain damage. Familial forms exist and have an autosomal recessive inheritance pattern (Moreno et al. 1989).

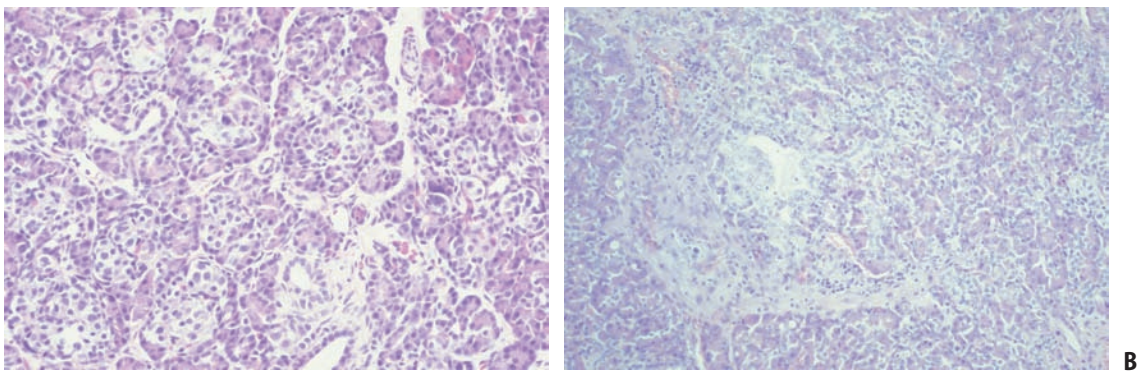
Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) is a hyperfunctional disorder of pancreatic insulin-producing cells, and recent advances in molecular genetics have shown a well-defined molecular basis for the disease (Reinecke-Lüthge et al. 2000).

Three basic genetic defects have been delineated: the first (autosomal recessive) results from a loss-of-function mutation on chromosome locus 11p15.1, which affects genes responsible for the adenosine triphosphate (ATP)-dependent potassium channel essential for the apparatus of insulin release; the second (autosomal dominant) results from a gain-in-function mutation of genes (*GLUD1* gene or the *GCK* gene) responsible for different enzymes regulating the rate of insulin release; the third is a loss of heterozygosity (LOH) in a group of β-cells with specific loss of maternal alleles of the imprinted chromosome region 11p15.1, unmasking a paternally inherited recessive.

These three genotypes correlate with the phenotypes. The autosomal recessive is associated with diffuse B-cell hyperplasia of early severe onset; the autosomal dominant is also a diffuse B-cell disease, but milder and of later onset. The LOH mutation is found in the focal form of the disease.

In the majority of cases of persistent hyperinsulinemic hypoglycemia there is a morphological as well as a functional abnormality of the endocrine pancreas. This is commonly called nesidioblastosis (Laidlaw 1938). Because endocrine malfunction appears more important than morphology, other terms, such as *nesidiodysplasia* (Gould et al. 1983) and *endocrine cell dysplasia* (Jaffe et al. 1980) have been suggested. Despite their logic, neither term has been widely accepted to replace nesidioblastosis. Three forms of nesidioblastosis are described: diffuse, focal, and, rare in infancy, adenoma.

In diffuse nesidioblastosis the pancreas is macroscopically normal. Histological examination shows an apparent increase in both islet and nonislet endocrine tissue. There is usually nuclear pleomorphism and gigantism of the insulin-producing B cells indicating hyperfunction (Fig. 24.36A). Ductulo-insular complexes may be hyperplastic with squamous metaplasia of ductular epithelium (Fig. 24.36B). Immunocytochemistry demonstrates the proportion and location of all endocrine cell types; B cells may predominate. Not all cases of diffuse nesidioblastosis show hypertrophy of B cells and can thus be very difficult to distinguish from the normal perinatal pancreas with its high proportion (10%) of endocrine cells. Even quantitative studies (Jaffe et al. 1980; Gould et al. 1983) comparing total endocrine tissue and B cells in the pancreas of infants with



A B

Figure 24.36. Pancreas, neonate with persistent hypoglycemic hyperinsulinism. (A) Nuclear gigantism of both islet and nonislet endocrine cells. (H&E, ×130) (B) Hyperplastic ductuloinsular complex with squamous metaplasia and pleomorphism of endocrine component. (A,B courtesy of Dr. A.G. Howatson, Glasgow, Scotland.) (H&E, ×130)

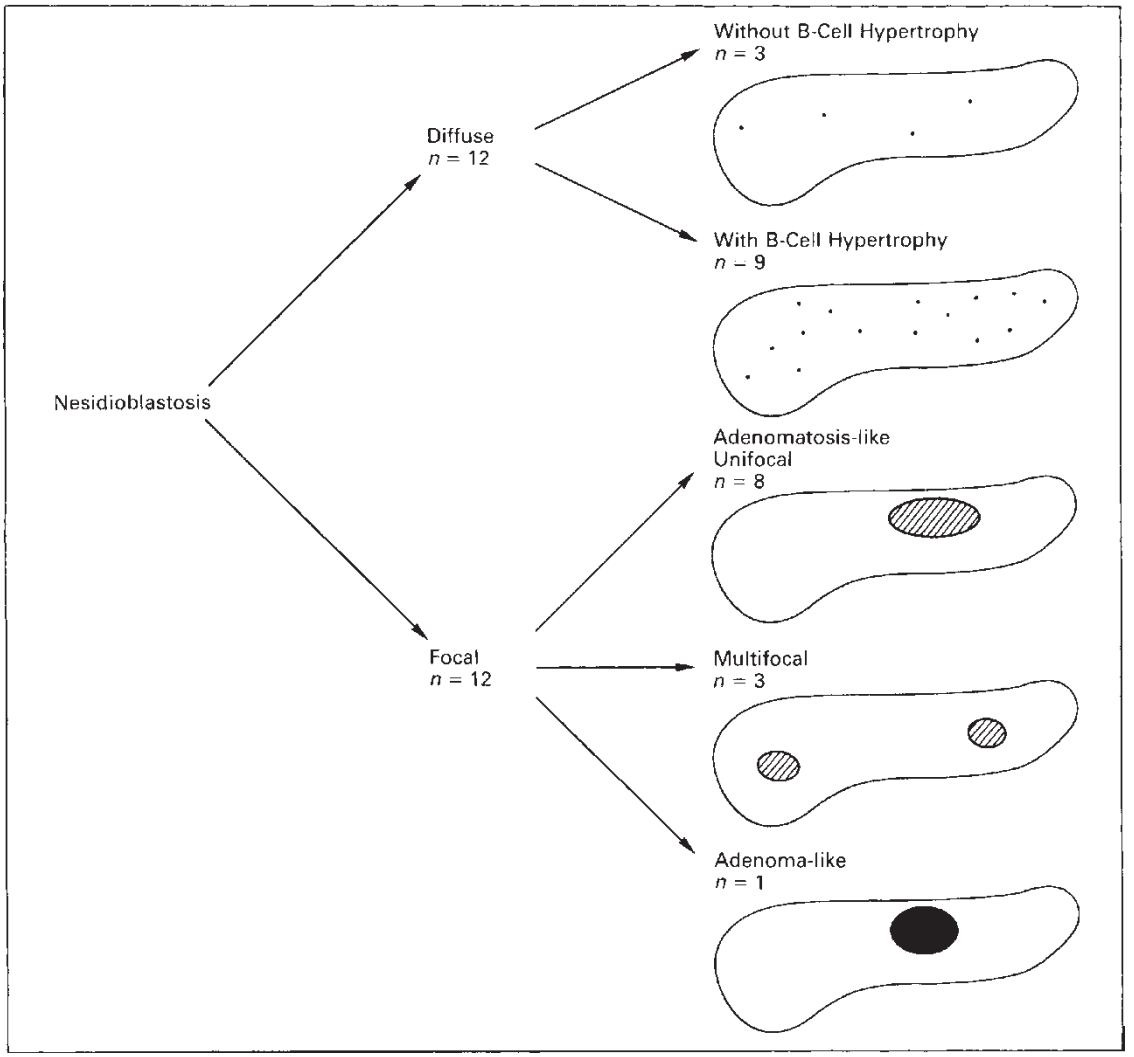


FIGURE 24.37. Distribution of focal and diffuse nesidioblastosis in 24 infants with persistent neonatal hyperinsulinemic hypoglycemia. (From Goosens 1989.)

persistent hypoglycemia with normal controls found that the endocrine mass was not invariably higher in hypoglycemic patients.

Focal nesidioblastosis and discrete adenomas are less of a diagnostic problem. One or more pale nodules 0.5- to 1.0-cm diameter may be visible. In focal adenomatosis, 40% of the affected area may comprise endocrine cells. They may be so densely packed that admixed exocrine acini and ducts may be difficult to see and the lesion mimics a true adenoma. Within focal nesidioblastosis the B cells may be very large with nuclear gigantism. Although all endocrine cell types are present, insulin cells predominate. In true adenoma, no exocrine pancreas is present. It is composed entirely of B cells separated by fibrous septa. In a Belgian study (Goossens et al. 1989) of 24 neonates with hyperinsulinemic hypoglycemia, three infants showed no B-cell hypertrophy, nine showed diffuse nesidioblastosis, and 12 had focal lesions (Fig. 24.37).

In all cases of persistent hyperinsulinemic hypoglycemia, in which any form of nesidioblastosis is suspected, immunohistochemical stains for insulin and other peptides should be carried out on any pancreatic tissue excised.

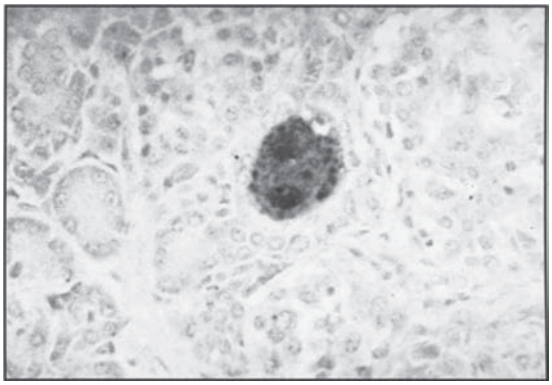


Figure 24.38. Pancreas, stillbirth. CMV inclusion in islet cell. (PAP, ×1200)

Rare causes of persistent neonatal hypoglycemia are hypopituitarism, GH deficiency, hypomagnesia, and glycogenosis types I and II.

Infection

Cytomegalovirus, mumps virus, and the Coxsackie virus group can all infect the endocrine tissue. In CMV, typical "owl's eye" viral inclusions can be seen in islet cells (Fig. 24.38), but no inflammatory response is elicited and destruction of the islets does not appear to occur.

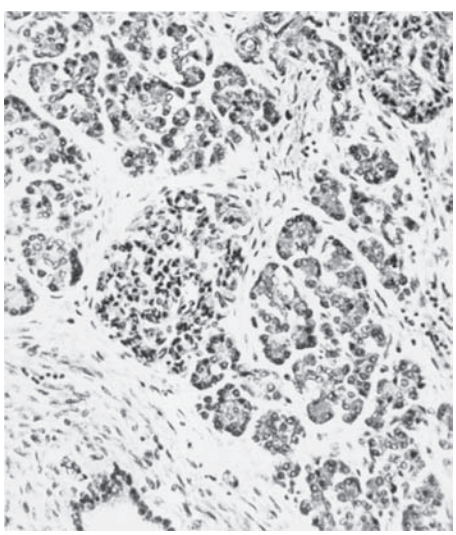
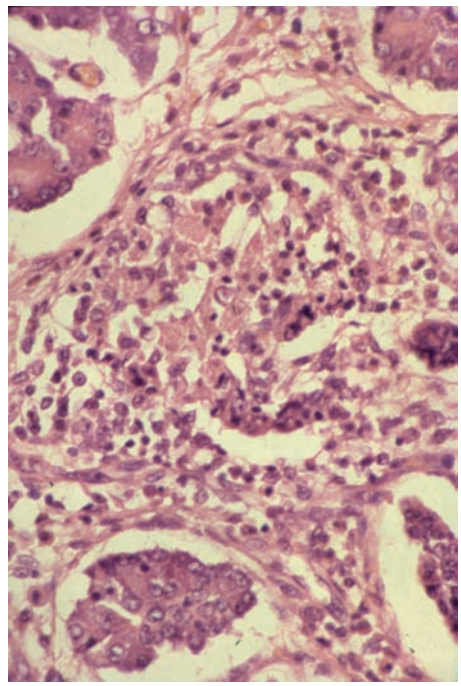


Figure 24.39. Pancreas, neonate, 3 days old. Islet cells show eosinophilic shrinkage of cytoplasm, nuclear pyknosis and fragmentation. Probable Coxsackie infection. (Courtesy of Dr. A. King, Cambridge, England.) (H&E, ×125)



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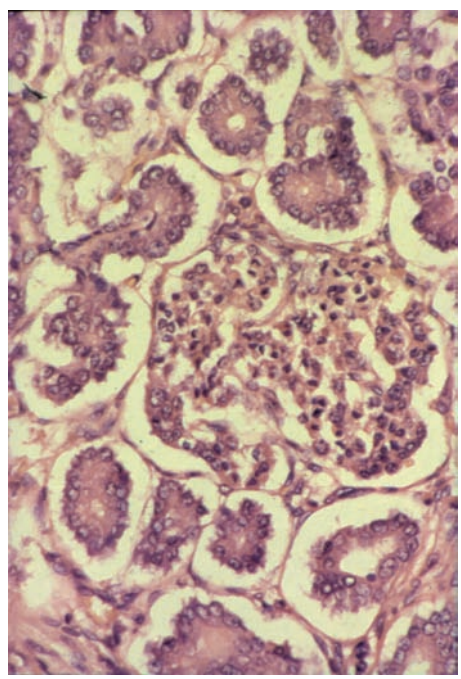

B

Figure 24.40. Pancreas, neonate, 9 days old, with severe Coxsackie B3 infection. (A) Interstitial inflammatory infiltrate; severe necrosis of islets *(arrow)*. (H&E, ×125) (B) Eosinophilic necrosis of islet cells, infiltrate of lymphocytes and eosinophils (arrows). (A,B courtesy of Dr. A. King, Cambridge, England.) (H&E, ×200)

Mumps has not been shown to produce any specific lesion, but Coxsackie B viral infections do produce islet damage and for this reason have caused much interest as a possible environmental factor in the onset of diabetes mellitus.

Coxsackie B infections in neonates usually present as myocarditis, but occasionally there is involvement of the exocrine and endocrine pancreas. The histological picture is quite variable, but the characteristic feature is necrosis of many of the islet cells, the cytoplasm being intensely eosinophilic and the B-cell nuclei pyknotic or even fragmented. The diagnosis is easy to miss if there is not a significant accompanying inflammatory infiltrate (Fig. 24.39).

In our own case there was a generalized sparse interstitial lymphocyte infiltrate, but around the necrotic islets were numerous eosinophils (Fig. 24.40). Eosinophils are not described in a previous case (Yoon et al. 1979). The cells destroyed are thought to be B cells; this was demonstrated in our case by immunohistochemical studies, which showed total loss of insulin-containing cells.

The possible role of such viral infection of the endocrine pancreas in triggering insulin-dependent diabetes mellitus is controversial, but there are human and animal studies implicating both mumps and Coxsackie. This topic as well reviewed by Kloppel (1984).

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25
The Reticuloendothelial System

T. Yee Khong

This chapter focuses on the morphological aspects of the spleen and thymus. Maternal and neonatal hematological problems that can contribute to adverse pregnancy or neonatal outcome are discussed in Chapter 8.

Spleen

Normal Development

The spleen is derived from mesenchymal cells found between the layers of the midline dorsal mesentery (mesogastrium) between the aorta and stomach, developing in the 5th week. Rotation of the stomach and growth of the mesentery results in the left-sided location of the spleen. The left surface of the dorsal mesentery fuses with the peritoneum overlying the left kidney. These events leave a lienorenal ligament between the kidney and the spleen and a gastrosplenic ligament between the spleen and the stomach. It is lobulated in the fetus, but the lobules are lost by term, being represented throughout life by notches or clefts on the superior border. Hemopoiesis begins in the organ at about 12 weeks and lymphoid follicles appear at 22 to 24 weeks (Figs. 25.1 and 25.2). Splenic weight increases 10-fold in the second half of pregnancy. Gestation related normal weights are tabulated (Table 25.1).

Developmental Anomalies

The term *polyasplenia* has been coined to encompass the spectrum of disorders of asplenia and polysplenia (Opitz 1985). There is commonality of malformations in other organ systems, and both asplenia and polysplenia have been described in the same family.

Asplenia is usually accompanied by major abnormalities of the cardiothoracic and abdominal organs, including right atrial isomerism, trilobation of both lungs, and bilateral eparterial bronchi (Ivemark's syndrome) (Ivemark 1955) (Table 25.2). Anomalous pulmonary venous drainage, persistent left superior vena cava, and atrioventricular abnormalities may also be present. The cardiovascular anomalies in this syndrome are complex and highly variable (see Chapter 21). This syndrome is overrepresented among hydropic fetuses with cardiovascular anomalies (see Chapter 14), and malrotation of the intestines or malpositioning of the viscera is also seen. Isolated congenital asplenia is rare, with only about 65 reported cases (Halbertsma et al. 2005) and can even be familial (Gilbert et al. 2002).

An uncommon association of asplenia is horseshoe adrenal gland but it is seen in over 50% of horseshoe adrenal gland (Strouse et al. 2002). Fused adrenal gland and anal atresia or stenosis are exclusive to asplenia of the heterotaxy syndromes (Ticho et al. 2000). Other midline defects in heterotaxy syndromes include cleft palate and absent corpus callosum (Noack et al. 2002).

In addition to multiple spleens in the dorsal mesogastrium, polysplenia is associated with other abnormalities—left atrial isomerism, bilateral hyparterial bronchi, and bilobed lungs—and complex and unpredictable visceral arrangements

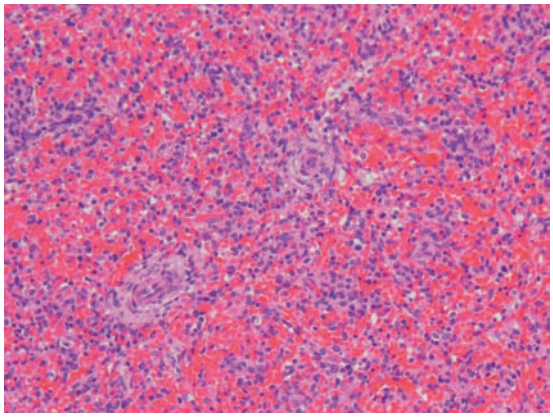

Figure 25.1. Spleen at 18 weeks with red pulp and arteriole devoid of periarteriolar lymphocytes.

in the abdomen are found. Cardiovascular anomalies are frequent but not as severe as those accompanying asplenia. The splenic mass is usually divided into fairly equally sized masses that together equal the mass of a normal spleen.

Accessory spleens (splenunculi) are seen in 10% of postmortems, most commonly adjacent to the tail of the pancreas or the hilum of the definitive spleen. One or more splenic masses are seen around a normally sized spleen; accessory spleens differ from polysplenia by the absence of the visceral anomalies seen in the latter.

Splenogonadal fusion (SGF) is an uncommon anomaly, and two types have been proposed. In the continuous type, the spleen is connected to the gonad and often it is associated with limb defects

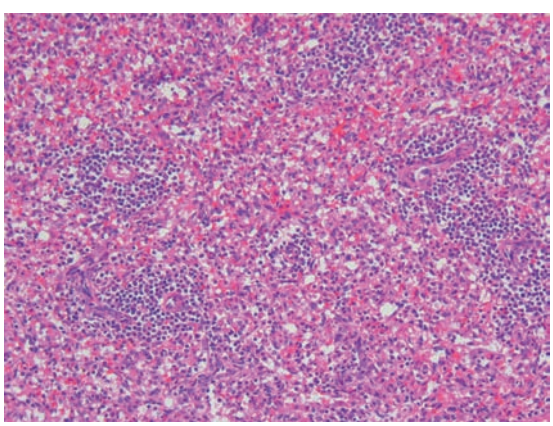

Figure 25.2. Spleen at 25 weeks with lymphocytic cuffing of arterioles.

Table 25.1. Spleen and thymus weights at different gestations (Gruenwald and Minh 1960)

Gestational age (weeks)	*n*	Spleen weight (g)	SD	Thymus weight (g)	SD
24	108	1.7	1.1	2.7	1.4
26	143	2.2	1.5	3.0	2.3
28	139	2.6	1.4	3.8	2.1
30	148	3.4	2.0	4.6	2.3
32	150	4.1	2.1	5.5	2.3
34	104	5.2	2.1	7.5	3.8
36	87	6.7	3.0	8.1	4.2
38	102	8.8	4.2	9.7	4.8
40	220	10.0	3.9	9.5	4.4
42	112	10.2	4.3	10.4	4.4

and other anomalies such as micrognathia, microglossia, anal atresia, and hypoplastic lungs (Fig. 25.3). Associated abnormalities are lower in the discontinuous type of SGF, where there is fusion between the gonad and accessory splenic tissue, without connection with the normal spleen. The cause of SGF and SGF with limb defects (SGFLD) is unknown, although they may result from a

Table 25.2. Classification of asplenia

Congenital	
Isolated	
Familial	
Nonfamilial	
Syndromic	
Ivemark	
Stormorken	AD; thrombocytopenia, muscle contractile defect, migraine-like headache, miosis, dyslexia, and ichthyosis
Kartagener	AR; ciliary dyskinesia, situs inversus
Meckel	AR; encephalocele, postaxial polydactyly, cleft palate, cystic dysplastic kidneys, hepatic portal fibrosis
Pallister-Hall	
Microgastria-limb reduction defects	
Smith-Fineman-Meyers	Mental retardation, short stature, cryptorchidism
Schmidt	
Crawfurd	AR; cardiac malformations, cystic dysplastic kidneys pancreatic cysts and fibrosis, hepatic portal fibrosis
Acquired	
Splenectomy	
Functional/hyposplenism	

AD, autosomal dominant; AR, autosomal recessive.

Figure 25.3. Splenogonadal fusion with splenic tissue present in continuity with the gonad through the inguinal canal. (Courtesy of Dr. J. Keeling, Edinburgh, Scotland.)

developmental field defect that originates during blastogenesis. Although the cause is unknown, the earlier its action, the more severe the involvement resulting in SGFLD; later action may result only in SGF (Bonneau et al. 1999; McPherson et al. 2003).

Splenopancreatic fusion is an uncommon finding and related usually to trisomy 13 (Hashida et al. 1983), but it has been described also in trisomy 21 and with other unrelated congenital anomalies (Peres et al. 2004). Three possible anatomical relationships may be seen: ectopic splenic tissue present in the tail of the pancreas; ectopic pancreatic tissue present in the spleen or an accessory spleen; and fusion of the tail of the pancreas with the hilum of the spleen (Fig. 25.4). The intrapancreatic tissue is poorly encapsulated or nonencapsulated, while the intrasplenic pancreatic tissue contains pancreatic acini and islets.

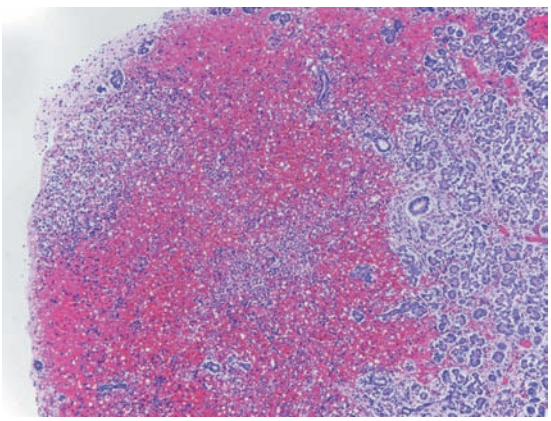

Figure 25.4. Splenopancreatic fusion showing expansion of pancreas by splenic tissue in a fetus with trisomy 13. (Courtesy of Dr. A. Charles, Perth, Australia.)

Wandering spleen or ectopic spleen refers to migration of the spleen from its normally fixed location in the left upper quadrant. Failure of development of the lienorenal and gastrosplenic ligaments results in a long-standing mesentery and abnormally mobile spleen. The anomaly is rare but has been reported in prune belly syndrome where there is a deficient anterior abdominal wall musculature (Teramoto et al. 1981).

Splenomegaly

In the fetal and neonatal period splenomegaly is most often due to congenital infection, chronic hemolysis, or accumulation of abnormal metabolites in storage disorders such as Gaucher's disease, Niemann–Pick disease, and mucopolysaccharidoses. In congenital leukemia it is usually accompanied by generalized lymphadenopathy.

In severe hemolytic disease of the newborn the spleen contains iron pigment deposits from excessive red cell breakdown and prominent hemopoiesis obscuring the red and white pulp.

Neoplasms

Hemangioma, although rare, is the most common primary tumor of the spleen. Cases have been reported in the neonatal period (Lee 2002). In the pediatric period, splenic hemangioma have been reported in association with Beckwith-Wiedemann syndrome and with Turner syndrome (Castriota-Scanderbeg et al. 1997; Herman et al. 1997).

Thymus

Normal Development

The thymus is derived from the ectoderm, endoderm, and mesenchyme of the 3rd and 4th pairs of pharyngeal pouches. Some of the ectodermal cells coalesce to form Hassall's corpuscles, the endoderm forming a reticulum with mesenchyme, which is colonized by $CD3^+$ prothymocytes from yolk sac and liver. Differentiation of these cells within the thymus is influenced by the products

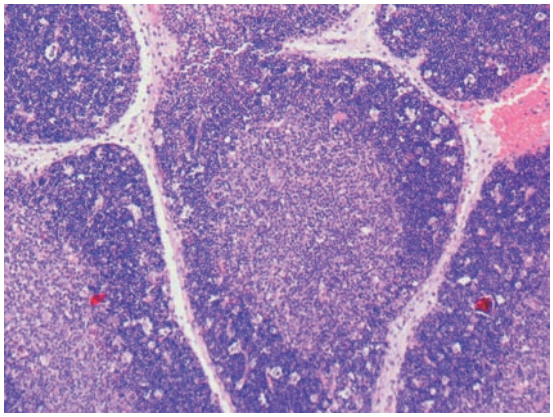

Figure 25.5. Early stress changes in thymus: corticomedullary demarcation is evident but cortical starry-sky pattern is present.

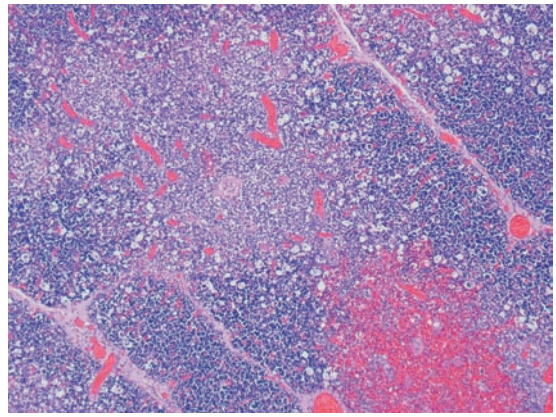

Figure 25.6. Stress changes with petechial hemorrhage and moderately extensive lymphophagocytosis leaving a starry-sky appearance.

of the thymic epithelium and the expression of surface markers changes during gestation. Relatively weak antigen-specific cytotoxicity is present as early as 12 weeks, but T-lymphocyte interactions with antibody-forming B cells are characterized by marked suppressor activity, possibly contributing to reduction of maternal immune responses to fetal antigens.

The developing gland descends into the thorax as the heart and lungs develop, though a variable cervical portion persists. The two separate lobes fuse about 50 days after conception and, histologically, corticomedullary distinction is apparent between 11 and 14 weeks; Hassall's corpuscles appear between 12 and 16 weeks (Fig. 25.5). The clear demarcation between cortex and medulla seen in the latter second trimester is blurred by term. The thymus is relatively prominent during the perinatal period and infancy but, by puberty, undergoes physiological involution by apoptosis.

Pathological Thymic Involution

Stress during intrauterine life leads to involution of the thymus through apoptosis of most lymphocytes and exodus of a few lymphocytes. van Baarlen and colleagues described four grades of involutional changes. Initially, some lymphophagocytosis is present in the cortex (grade 1), which then becomes more pronounced to expose the epithelial cells and give a "starry sky" appearance associated with shrinkage of the cortex and some separation of the thymic lobules (grade 2) (Figs. 25.5 and 25.6). In the next stage, further lymphophagocytosis leads to increasing loss of the corticomedullary demarcation and increasing separation of the lobules (grade 3) (Fig. 25.7) until finally there is such marked depletion of the cortical lymphocytes that the lymphocyte density is greater in the medulla than in the cortex and there is shrinkage and separation of the thymic lobules (grade 4). Stress-related involution progresses with increasing duration of stimulation and appears to be reversible. Increasing duration of stress is correlated with a decrease in thymic weight and histological extent. The latter

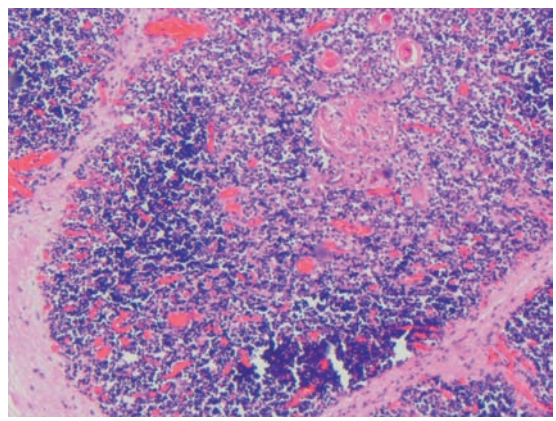

Figure 25.7. Increased fibrous separation of thymic lobules associated with an almost total loss of corticomedullary demarcation and prominent Hassall's corpuscles.

correlation is approximate, but by and large no changes were observed with duration of disease of less than 12 hours; <80% with a starry-sky pattern (grade 2) will be seen within 24 to 48 hours while >80% of cases with grade 4 histology had disease of longer than 72 hours' duration. Not surprisingly, weight is lowest in thymuses showing the largest extent of involution.

The appearances seen in the involutional thymus may be difficult to distinguish from those seen in many immunodeficiency syndromes.

Thymic Aplasia and Hypoplasia

Absence of the thymus was first described in 1929, and the association with hypoparathyroidism was first described in 1959, but it was not until 1967 that DiGeorge proposed that the immunological deficiencies resulting from the thymic aplasia and hypoparathyroidism were due to defects in the development of the third and fourth pharyngeal pouches (Di George et al. 1967). Since then, two other overlapping syndromes, viz. velocardiofacial syndrome and conotruncal anomaly facial syndrome, have been described with deletions in the region of chromosome 22q11 as a common feature, thus defining 22q11 deletion syndrome or the acronymic CATCH-22 syndrome (*c*ardiac defects, *a*bnormal facies, *t*hymic hypoplasia, *c*left palate, and *h*ypocalcemia) (Wilson et al. 1993). In velocardiofacial syndrome, there are typical facial features, cleft palate, learning disabilities, and specific congenital heart defects. Conotruncal anomaly face syndrome was defined by conotruncal cardiac defects, hypernasal speech, mild mental retardation, neonatal tetany, thymic aplasia or hypoplasia, and facial dysmorphism. It is likely that mutation of *TBX1,* encoding a T-box transcription factor, results in the observed phenotype (Yagi et al. 2003), but whether other genes in the region also contributes to the phenotype remains unanswered.

Using fluorescence in-situ hybridization, two population-based studies found a prevalence of about 14 per 100,000 live births in Sweden and 17 per 100,000 live births in the United States; both research groups stressed that the rates were dependent on the severity of the phenotype and clinical awareness of the syndrome (Botto et al. 2003; Oskarsdottir et al. 2004). The sexes are affected equally. Features common to the 22q11 deletion syndrome are congenital heart disease, immunodeficiency, hypocalcemia, palate anomalies, velopharyngeal dysfunction and other speech disorders, feeding disorders and growth restriction, otorhinolaryngology issues, dysmorphic facies, renal anomalies, skeletal anomalies, and neurocognitive disorders (Goldmuntz 2005).

The thymic aplasia or hypoplasia results in severe defective T-cell function as a result of the absence of processing of stem cells to T cells, with resultant susceptibility to infection with fungi, viruses, and *Pneumocystis*. Paracortical areas of lymph nodes are depleted; B-cell numbers are normal. Thymic hypoplasia or aplasia has been described in aneuploidies other than 22q11 deletion, including partial monosomy 10p, trisomy 18, trisomy 14q, and trisomy 22 (Chen et al. 1979; Tovo et al. 1986; Ohtake et al. 1994; de Pater et al. 2005; Schuffenhauer et al. 1995). In trisomy 21, there is often a functional deficiency of T cells, and the morphology of spleen, lymph nodes, and thymus reflects this, with indistinct splenic periarteriolar sheaths, depletion of lymphocyte numbers in paracortical areas in nodes, and a small thymus with relatively few cortical lymphocytes. Hassall's corpuscles may be enlarged, calcified or cystic (Murphy et al. 1993).

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26
Malformations of the Nervous System and Hydrocephalus

Colin Smith

Development of the central nervous system (CNS) is remarkably complex and only now are we beginning to unravel the detailed mechanisms underlying the morphological processes that have been described by embryologists for decades. That this complex process should on occasion fail should not be surprising; that the process proceeds in a normal fashion in most conceptions is a reminder of the wonderful complexities of nature.

The actual incidence of CNS malformations is difficult to assess, as CNS development begins within 15 days of conception. Malformations at this stage are likely to be lethal, resulting in embryo death. However, it has been estimated that up to 10% of stillbirths are affected by a CNS malformation and that about 5% of early neonatal deaths are related to CNS malformations (Harding and Copp 2002).

Malformations are the result of disruption of the normal developmental process. Therefore, malformations can only develop either before or during the developmental process. When the structure has passed through its developmental stage, insults will result in damage to the tissue with associated cellular responses. This, however, is not a malformation but rather an acquired injury. Such injuries are the subject of Chapter 27.

The etiology of CNS malformations is unknown in most cases. However, a genetic basis has been described in some cases, including single gene defects that may result in loss or gain of function, and chromosomal abnormalities. Environmental causes include infections and drugs, both recreational and prescription. Maternal factors, both genetic and acquired, may also interrupt the developmental process.

This chapter outlines key developmental processes and some of the CNS malformations encountered.

Early Central Nervous System Development

Central nervous system development begins within 2 weeks after conception, and by 18 days a neural plate can be seen within the embryonic ectoderm. The bilaminar embryonic disk, composed of an upper epiblast and lower hypoblast, becomes trilaminar by the process of gastrulation. The trilaminar embryo is composed of, from top down, epiderm, mesoderm, and endoderm. Neural induction is the process by which regions of the ectoderm are established as the precursors of the future nervous system. The process of induction is complex, and while significant gains have been made in our understanding of this process in animal models there are still unanswered questions (Stern 2005). Animal models have highlighted that modulation of the expression of bone morphogenetic proteins (BMPs) is essential for normal neural induction. The BMP levels may be modulated at various stages of neural induction by secretion of BMP antagonists such as noggin and chordin, allowing the expression of neural commitment genes such as the *Sox* family of genes encoding transcription factors (Sasai 2001). The

prechordal plate and notochord are important inducers, regions from which chemical signals are released, but do not act alone, with surrounding nonneural ectoderm also being important in the process (Stern 2001).

Neural induction is followed by neurulation, the process by which the neural plate folds and closes to form the neural tube. Neurulation can be separated into primary neurulation, which describes the folding and fusion of the neural plate, and secondary neurulation, which occurs caudally with condensation of mesenchymal cells in the caudal eminence (tail bud). Primary neurulation requires several steps for successful completion: cell proliferation and movement, folding of the neural plate, and fusion. Cell proliferation and movement results in shaping of the neural plate with a broad rostral and narrow caudal region. The neural plate then begins to fold into the neural tube at around days 17 to 18. Folding requires changes in cell shape at three key regions: the median hinge point overlying the notochord, and two dorsolateral hinge points (Sadler 2005). The median hinge point extends the entire length of the neural tube and is induced by sonic hedgehog (Shh) protein, released from the notochord; Shh inhibits formation of the dorsolateral hinge points such that they are not present at upper spinal cord regions but are seen in cranial and lower spinal regions where Shh concentrations are lower. These hinge points allow bending through changes to internal cell structure and changes to the normal interkinetic nuclear migration. There is an accumulation of cells with basally situated nuclei and reduced apical surface area. Contraction of actin filaments in the apical region of these hinge cells further assists folding, as does the action of surrounding non-neural ectoderm.

Fusion of the neural tube follows folding and occurs in a rostrocaudal fashion. Fusion is initiated at three separate sites: initially at the hindbrain–cervical cord region, followed by the forebrain-midbrain region, and at the rostral end of the forebrain. From these sites fusion extends in both a rostral and caudal direction, culminating in closure of the anterior and posterior neuropores at approximately days 25 and 27, respectively (Fig. 26.1). The molecular mechanisms and adhesion molecules involved in fusion are not well defined, but ephrin proteins and Eph receptors may be important (Copp et al. 2003). The fused neural tube then separates from the nonneural ectoderm.

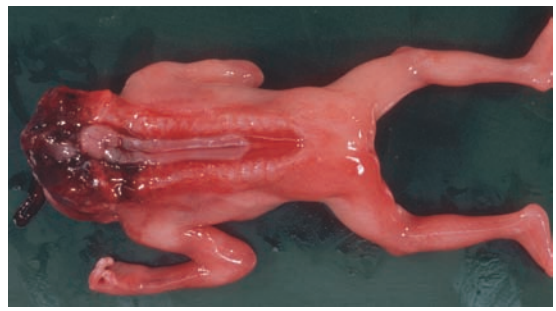

Figure 26.1. An 18-week fetus with craniorachischisis. There is complete failure of neural tube closure, exposing the spinal cord. There has been no fusion of the rostral tube, resulting in abnormal development of and damage to the forebrain structures, which are replaced by hemorrhagic tissue.

The resulting neural tube shows a range of patterning differences in both the dorsoventral and rostrocaudal axes. Dorsoventral patterning separates the brainstem and spinal cord into ventral motor regions and dorsal sensory regions. Dorsalizing factors are predominantly BMPs, which are released from the roof plate of the neural tube (Chizhikov and Millen 2004). Ventralizing factors are predominantly Shh proteins from the notochord and floor plate of the neural tube (Jessell 2000). Rostrocaudal patterning is discussed later in relation to cerebral development.

Malformations Related to Early Central Nervous System Development

Neural Tube Defects

Neural tube defects (NTDs) are among the commonest malformations of the CNS. They range from small focal defects of the vertebrae of limited clinical significance (spina bifida occulta) through to extensive defects incompatible with life (anencephaly and craniorachischisis). Neural tube defects can be described as being either open, in which the abnormal neural tube is exposed to the outside world through an epidermal defect, or closed, in which the overlying epidermis is intact. Open defects are a result of failure in primary neurulation mechanisms, while closed defects are

a consequence of secondary neurulation defects (Copp et al. 2003). Closed defects are rarely seen in fetal or neonatal practice and are not discussed further.

There is considerable geographic variation in the incidence of NTDs, ranging from 0.1 per 1000 live births in low-risk areas to 12.5 per 1000 live births in high-risk areas (Shurtleff and Lemire 1995). The incidence of NTDs has been reduced by the introduction of dietary folate supplementation at the time of conception (Wald et al. 1991). The NTDs can be screened for during pregnancy, with serum α-fetoprotein (AFP) screening and detailed ultrasound examination in "at-risk" cases.

Craniorachischisis and Anencephaly

Craniorachischisis and anencephaly represent the most extensive of the neural tube defects. In craniorachischisis the neural tube from the midbrain down to the upper sacral region of the spinal cord remains open (Fig. 26.1), there being no fusion of the tube. This represents failure of primary fusion at the hindbrain–cervical cord region. If hindbrain–cervical cord fusion proceeds normally but there is failure of fusion at the subsequent two rostral initiating sites described above, anencephaly develops. Macroscopically the exposed nervous tissue is necrotic due to exposure to amniotic fluid. An anencephalic fetus has a typical appearance (Friede 1989a). The orbit is partially formed, often being shallow, with bulging eyes. The cranial vault is absent (acrania) and the skull base flattened, with the sphenoid bone having an appearance likened to "a bat with folded wings." The posterior fossa is normally funnel-shaped, although a subclassification of anencephalic fetuses has been proposed based on the radiological appearances of the posterior fossa; some anencephalic fetuses had a morphology close to normal while others had a small malformed posterior fossa (Lomholt et al. 2004). The defect in the cranium is filled by a mass of reddish tissue, the area cerebrovasculosa. As the name suggests, this tissue is highly vascular and composed of a disorganized mass of neural and mesenchymal tissue. The neural tissue is composed mostly of glial tissue with scattered neurons and ependymal canals (Bell and Green 1982). Normally it is not possible to identify cerebellum or rostral brainstem beyond the medulla. As noted above, spinal cord involvement is variable.

Myelomeningocele

Myelomeningoceles are most commonly seen in the lumbar region, and many cases are associated with a Chiari type II malformation (see below). They result from failure of neural tube closure and abnormal development of the vertebral arches (Fig. 26.2) there being defects in the posterior aspects of the vertebrae (Fig. 26.3). Two main types are seen: meningoceles and myelomeningoceles. The meningocele has a closed neural tube but with cystic dilatation of the meninges through the absent vertebral arch. In myelomeningoceles the lesion is often flat and the neural tissue may lie in continuity with the skin or be separated from skin by a thin connective tissue (Fig. 26.4). The abnormal tissue is composed of vascular connective tissue intermixed with disorganized neural tissue (area medullovasculosa) including sections of peripheral nerves.

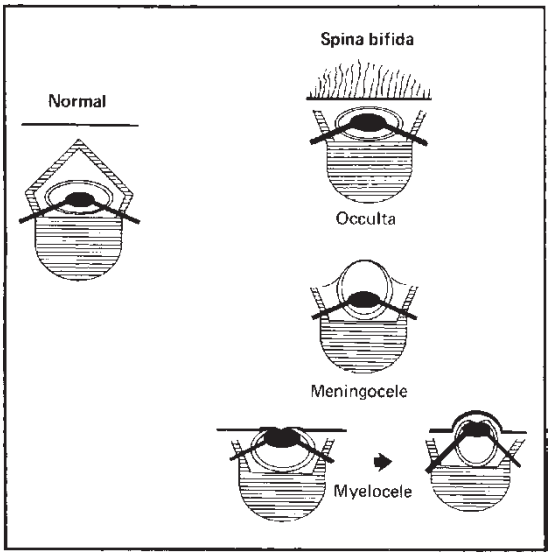

FIGURE 26.2. Diagrammatic representation of the anatomy of the normal spinal column and of spina bifida. In spina bifida there is a failure of closure of the vertebral arches, the cord being covered only by skin (spina bifida occulta), or meninges (meningocele). If there is also abnormal development of the cord, with failure of neural tube closure the lesion is a myelocele. The exposed cord may open onto the skin surface or be separated from the surface by connective tissue.

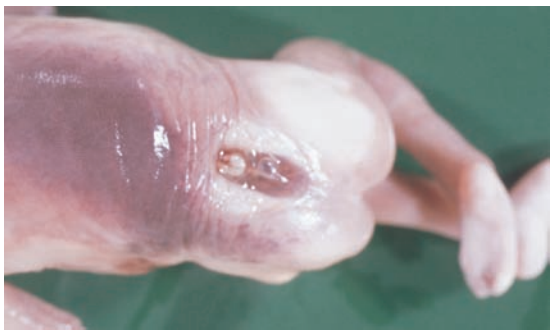

Figure 26.3. A lumbar myelocele, the skin, and the bony defect exposing the underlying spinal cord.

Much of the disability associated with myelomeningoceles is due to necrosis of nervous tissue caused by cerebrospinal fluid (CSF). In utero surgery before 24 weeks' gestation has been shown to reduce the neurological impairment at birth (Adzick et al. 1998).

Encephaloceles

Encephaloceles are caused by the herniation of brain or meninges through axial mesodermal defects (Copp and Harding 2004). The commonest site is the occipital region (70% to 80%), with frontal lesions being less common, although frontal encephaloceles are more common in Southeast Asia.

The encephalocele is a herniated sac, extending through a defect in the skull bones, covered by skin and dura (Fig. 26.5), although this may become ulcerated and infected. If no brain tissue is present in the sac, the correct term is *meningocele*. Meningoceles connect to the intracranial brain by either a broad base or, more commonly, a smaller stalk. Small encephaloceles often have disorganized fragments of nervous tissue, whereas larger encephaloceles may have sections of cerebral hemispheres and sometimes hindbrain in them. The herniated tissue is usually asymmetrical (Karch and Urich 1972), although in some cases tissue from both cerebral hemispheres will herniate. The herniated tissue may show a range of abnormalities including neuronal migration disorders.

An occipital encephalocele may be seen as part of Meckel-Gruber syndrome.

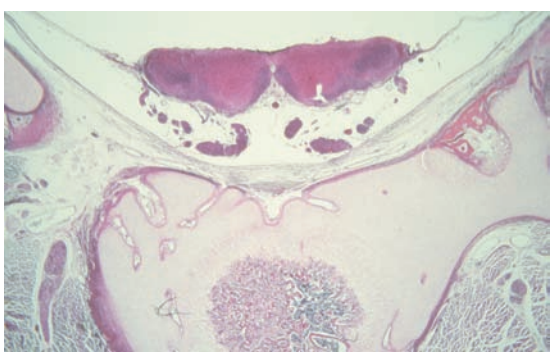

Figure 26.4. Photomicrograph of a myelocele demonstrating failure of fusion of the neural tube.

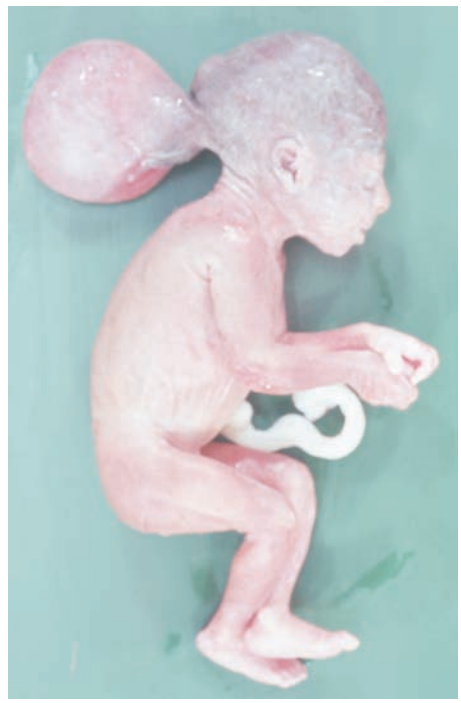

Figure 26.5. An occipital encephalocele, which, in this case, contained tissues from both cerebral hemispheres.

Disorders of Cerebral Development

During the 4th week of development the neural tube begins to bend as a result of variable growth rates resulting in flexures. These occur in the region of the midbrain and at the junction between the hindbrain and the spinal cord initially, and subsequently in the pontine region. At the same time, three brain vesicles form rostral to the

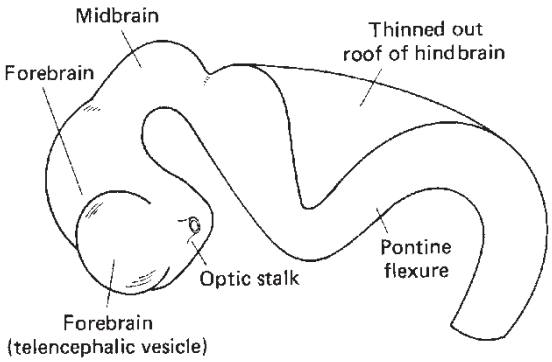

FIGURE 26.6. Rostrally flexures develop in the neural tube and vesicles develop from the forebrain, giving rise to the cerebral hemispheres.

developing cord: prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain) (Fig. 26.6). This section discusses the development of the prosencephalon.

As noted above the prechordal plate, notochord, and nonneural ectoderm release factors that play a role in the induction of neural tissue. At the same time other factors are released that are important in rostrocaudal patterning. Of particular importance to the development of the prosencephalon in some animal models are the anterior visceral endoderm and the expression of genes such as *Otx2* (Rallu et al. 2002).

During the 5th week the prosencephalon divides into a rostral telencephalon and a caudal diencephalon (secondary brain vesicles). The telencephalon gives rise to the cerebral hemispheres. The telencephalon develops as thin-walled vesicles with a thicker aggregation at the floor region, called the ganglionic eminence, composed of medial and lateral regions. The apparent cleavage of the telencephalon into paired vesicles is the result of slow midline growth compared to rapid lateral growth. This is in continuity with the diencephalon, which becomes enveloped by the telencephalic vesicles by 16 weeks. The cerebral cortex develops as a consequence of cellular proliferation, migration, and differentiation in the dorsal forebrain. Cellular proliferation begins at about week 8 and continues well into the 5th month. Cells proliferate in a region adjacent to the developing ventricular system, resulting in waves of neuroblasts moving from the ventricular region to the outer aspects of the developing cerebral cortex. Cerebral cortex formation follows an "inside-out" pattern, with successive waves of neuroblasts passing through already established cortical layers as they move to the outer aspect of the developing cortex, finally resulting in the six-layer mature neocortex. Early neurons that line the outer surface of the developing cortex, Cajal-Retzius cells, secrete a protein (reelin), which signals a stop signal to migrating neuroblasts.

Migration of the neuroblasts involves several mechanisms (Nadarajah and Parnavelas 2002), one of which is dependent on glial cells. Glial progenitors are also found in the ventricular proliferating zone but move into and populate the developing white matter at an early stage. Radial migration involves neuroblasts moving along radial glial fibers that guide them to the cortical surface. More recently, tangential migration has been described for interneurons arising in the ganglionic eminence, although the mechanisms involved in this process are to date poorly defined.

Abnormalities in development of the forebrain can be considered as either forebrain patterning defects or neuronal migration disorders.

Forebrain Patterning Defects

The most severe of the forebrain disorders are aprosencephaly and atelencephaly. As the terms suggest, aprosencephaly is a complete absence of the forebrain (telencephalon and diencephalon) resulting in a rudimentary forebrain structure being attached to the brainstem, while in atelencephaly the diencephalons can be identified. Both are rare disorders.

The commonest of the disorders of forebrain formation are the holoprosencephalies. Holoprosencephaly is the result of variable separation of the telencephalic vesicles, and macroscopically can be separated into alobar, semilobar, and lobar forms (Ming and Golden 2004). A majority of cases are sporadic, although familial cases are described. It may develop secondary to maternal infection or be a consequence of conditions such as maternal diabetes and fetal alcohol syndrome. It occurs as part of Smith-Lemli-Opitz, Pallister-Hall, and Rubinstein-Taybi syndromes, among others. At least 12 different genes involved in

holoprosencephaly have been described to date. These include *SHH*, *SIX3*, and *TGIF*. All are involved at various stages in forebrain specification and development.

In alobar holoprosencephaly there are characteristic craniofacial malformations, with facial hypoplasia and, in severe cases, cyclopia with a proboscis. The brain is a single small sphere with no interhemispheric fissure (Fig. 26.7). The gyral pattern is abnormal. Posteriorly the sphere is completed by a thin membrane that is continuous with the tentorium cerebelli (Fig. 26.8). There is a single ventricle, and in the base of the holosphere the thalami and basal ganglia are fused (Fig. 26.9). Microscopically there is cortical disorganization.

Semilobar holoprosencephaly differs in that there is a shallow interhemispheric fissure and identifiable lobar structure, usually with rudimentary occipital lobes.

In lobar holoprosencephaly the brain forms with a recognizable lobar architecture, but there is central fusion of the hemispheres (Fig. 26.10). This fusion may be anteriorly, centrally, or posteriorly (Fig. 26.11).

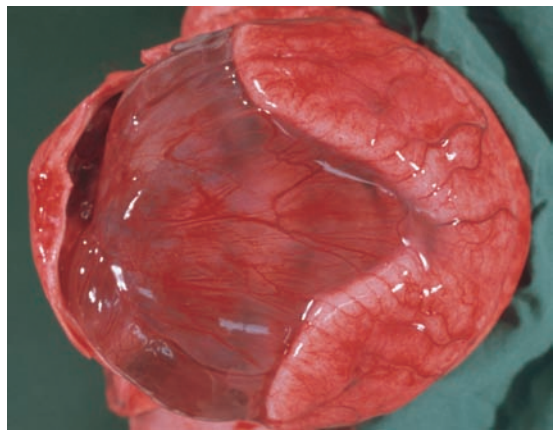

Figure 26.8. Alobar holoprosencephaly. Posteriorly the single sphere is completed by a thin membrane that is continuous with the tentorium cerebelli.

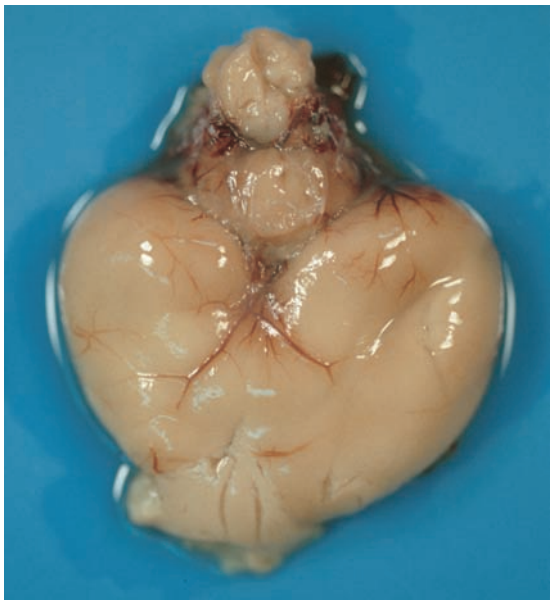

Figure 26.7. Alobar holoprosencephaly with the cerebrum being represented by a single sphere. There is no olfactory nerve development visible macroscopically.

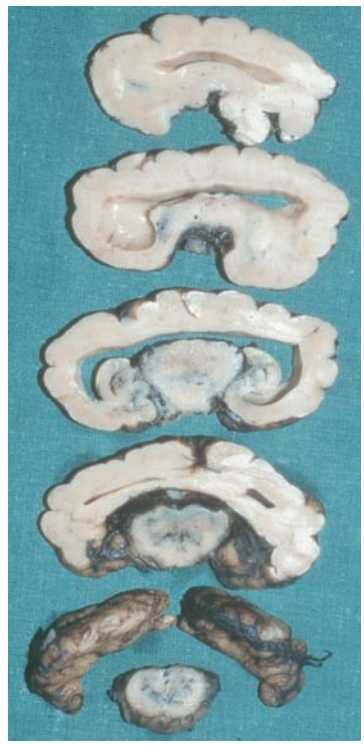

Figure 26.9. Coronal sections through the brain in a case of holoprosencephaly. There is a single ventricle with fused diencephalic structures.

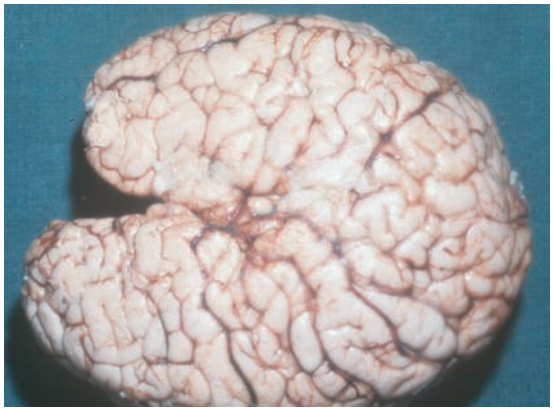

Figure 26.10. Lobar holoprosencephaly. The lobar structure of the brain is poorly developed and there is midline fusion of the hemispheres anteriorly.

Agenesis of the Corpus Callosum

Agenesis of the corpus callosum is a disorder of midline prosencephalic development. It can be either sporadic or as part of a familial disorder such as Aicardi syndrome. It may be the only malformation present, or may be associated with other malformations such as holoprosencephaly. Macroscopically, the abnormality can be partial or complete. In cases with partial agenesis, the defect is usually posterior, involving the splenium (an important exception being partial agenesis associated with holoprosencephaly).

At autopsy it can be difficult to decide if absence of the corpus callosum is a genuine defect or represents a postmortem artifact. In cases of true corpus callosum agenesis there is also absence of the cingulate gyrus, and an abnormal pattern of gyri radiating out from the medial surface of the hemisphere. On coronal sections the lateral ventricles have upturned pointed corners (Friede 1989b). White matter bundles (bundles of Probst) can be seen at the interhemispheric edge of the cortex running in a longitudinal direction (Fig. 26.12).

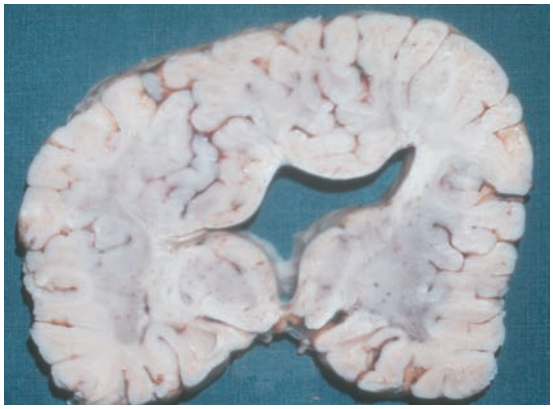

Figure 26.11. Coronal sections from a case of lobar holoprosencephaly. The cingulate gyri are fused and there is a single ventricular space.

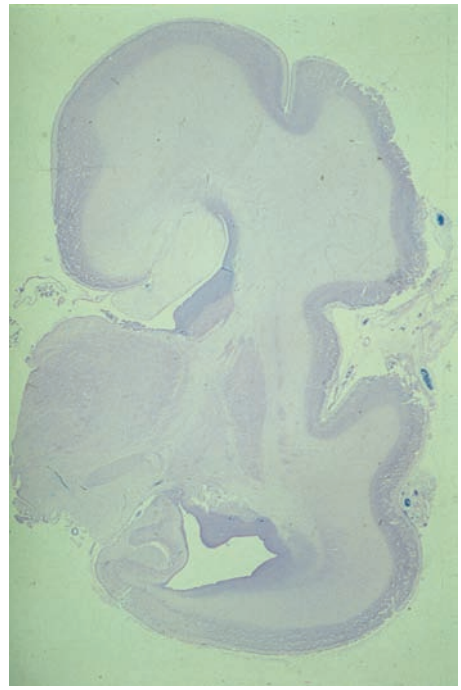

Figure 26.12. Coronal slice of a hemisphere of a 20-week fetus with absence of the corpus callosum. Note the bundle of Probst occupying much of the lateral ventricle and carrying a mass of fibers in an anterior-posterior direction.

Neuronal Migration Disorders

The normal process of neuronal migration is discussed above. This may be disrupted, leading to a range of abnormalities. In fetal and neonatal practice the most important group of disorders is the lissencephalies, although heterotopias may also be seen.

The term *lissencephaly* is of Greek derivation and means smooth brain. During normal development the brain is smooth prior to sulcal formation, which begins at approximately week 18. The

term *lissencephaly*, however, should be restricted to pathology, that is, cases in which the brain is abnormally smooth for the gestational period. Lissencephaly encompasses several different disorders with variable pathology and underlying genetic abnormality, and can be divided into type I (classical) and type II (cobblestone). As a group, the lissencephalies are uncommon, although published estimates of incidence are likely to underestimate the true number of cases.

Type I (Classical) Lissencephaly

To date, mutations in four genes have been described as underlying classical lissencephaly (Golden 2004a): *LIS1*, *XLIS*, *ARX*, and *RELN*. *LIS1* encodes the β-subunit of platelet-activating factor acetylhydrolase (PAFAH1B), *XLIS* encodes double cortin, *RELN* encodes for reelin, and *ARX* encodes a transcription factor. The role of these proteins in neuronal migration is slowly being defined (Gupta et al. 2002). PAFAH1B is thought to be important in interactions with the dynein motor system in the cell cytoplasm, a mechanism that is important in nuclear migration during mitosis and cellular migration. Reelin is an extracellular component of the outer molecular layer and is expressed by Cajal-Retzius cells. Reelin is required to bind to receptors on migrating neuroblasts as part of normal guidance during migration, although the precise function is poorly defined (Tissir and Goffinet 2003). The roles of double cortin, a microtubule-associated protein, and the *ARX* gene product, a transcription factor, remain unknown.

In the neonatal period classical lissencephaly may present with intractable seizures. Dependent on the underlying genetic abnormality there may also be variable dysmorphic features. In Miller-Dieker syndrome (*LIS1* mutations) hypertelorism and frontal bossing are seen, and congenital heart defects have been described.

In the *ARX* mutation cases there is hypoplasia of the adrenal glands and microphalus resulting in the XLAG syndrome (X-linked lissencephaly with ambiguous genitalia).

The macroscopic appearance of the lissencephalic brain is variable, due to the underlying genetic defect (Pilz et al. 2002), but in all cases there is paucity of gyri and sulci. In its severest form there is complete absence of sulci and gyri (agyria) (Fig. 26.13). This is seen in Miller-Dieker syndrome. Isolated lissencephaly sequence, due to less extensive *LIS1* mutations, usually shows more extensive loss of occipital gyri with relative frontal sparing. The reverse, with greater abnormality of the frontal lobes, is more suggestive of *XLIS* mutations. This pattern is also seen in *RELN* mutations, but with the addition of severe cerebellar hypoplasia. On sectioning, the areas of abnormal gyration show gross thickening of the cortex. A range of neuronal heterotopias may be seen.

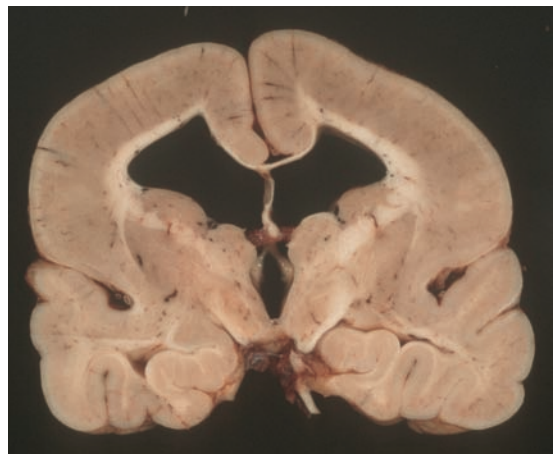

Figure 26.13. Coronal sections of a lissencephalic brain. The surface is smooth with gyral patterning only being seen on the inferior aspect of the brain.

Histologically, the normal six-layered neocortical architecture is replaced by a cortex showing variable abnormalities dependent on the underlying genetic defect (Forman et al. 2005). *LIS1* mutations show the classically described four-layered cortex, with disorganized pyramidal cells in layer 2 and thick layer 4 composed of small to medium cells.

Type II (Cobblestone) Lissencephaly

Most cases of type II lissencephaly have abnormalities of gyration associated with cerebellar, ocular and skeletal muscle abnormalities (Golden 2004b). Included in this group are Walker-Warburg syndrome, Fukuyama congenital muscular dystrophy, muscle-eye-brain disease, Fukutin disorders, and congenital muscular dystrophy type 1D. The underlying genetic mutations

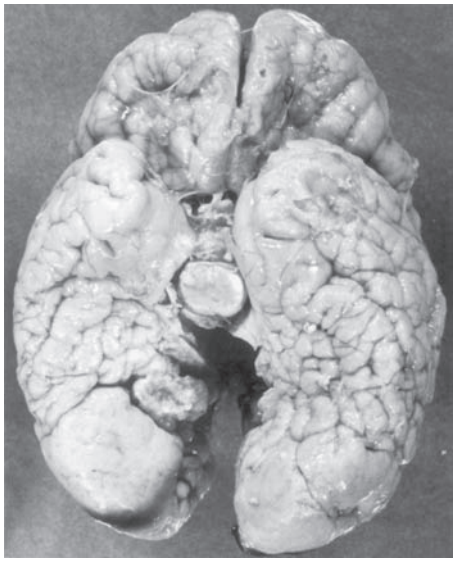

Figure 26.14. Lissencephaly from a case of muscle-eye-brain disease with a cobblestone appearance and agyria affecting the occipital lobes.

are all thought to involve abnormalities in glycosylation (Martin-Rendon and Blake 2003).

Walker-Warburg syndrome usually presents in the neonatal period with hypotonia. Macroscopically, the brains have a "cobblestone" appearance. As with type 1 lissencephaly the extent of the abnormality is variable, with Walker-Warburg syndrome showing the most extensive distribution (Fig. 26.14). On sectioning, the cortex is thickened in areas of abnormality. Histologically the cortex shows marked disorganization with no discernible laminar architecture and no paucicellular molecular layer. The migrating neurons appear to extend through the pial surface and colonize leptomeningeal space, incorporating leptomeningeal vessels into the abnormal cortical mass.

Cerebral Heterotopias

Disorders of neuronal migration may result in groups of neurons being located at atypical sites within the nervous system. Such heterotopias may be seen in isolation or, more commonly, in association with other malformations. They are classified on the basis of their position, with leptomeningeal, subcortical, and periventricular heterotopias being described (Monuki and Ligon 2004).

Leptomeningeal heterotopias are usually microscopic, the exception being the diffuse form seen in type II lissencephaly. Microscopically glioneuronal tissue is found in the leptomeninges, resulting in focal filling of the subarachnoid space. Lesions appear by 20 weeks' gestation and were consistently seen in trisomy 13 cases beyond 34 weeks' gestation (Iida et al. 1994).

Subcortical band heterotopias usually appear laminar macroscopically, although they may be nodular. The abnormal gray matter is clearly delineated from the overlying cortical tissue. Their extent is variable, ranging from bilateral and diffuse to unilateral and focal. They have a female predominance due to X-linked dominant inheritance. In most cases mutations of the *XLIS* gene have been described.

Periventricular heterotopias may be seen on coronal sections. They range from single nodules to extensive bilateral lesions (Fig. 26.15). They often push into the walls of the lateral ventricles, producing nodular distortion of the walls. They are associated with many syndromes, although the genetic basis is poorly understood in most. However, periventricular heterotopias are the main pathology in mutations of the *FLNA* gene, a lethal condition in males. The heterotopias can be difficult to identify in fetal brain due to the lack of myelination, and histologically the heterotopias may show appropriate neuronal maturation

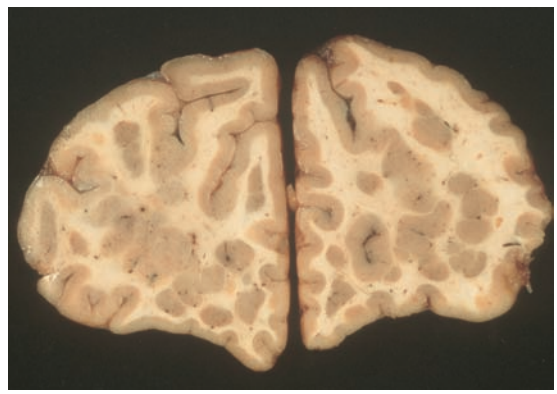

Figure 26.15. Neuronal heterotopias present bilaterally within the frontal lobes. Discrete nodules of gray matter are seen within the white matter. It is important not to confuse occasional cross-cut sections of cerebral cortex with neuronal heterotopias.

with attempted lamination. The overlying cortex is frequently dysplastic (Hannan et al. 1999).

Polymicrogyria

Polymicrogyria is a disorder of neuronal migration that can be both a malformation and an acquired disruption. It is defined as an area of the cortical ribbon with excessive folding. The thin folds of cortex may be fused or layered.

Familial cases of polymicrogyria are described, but to date no specific genetic abnormality has been defined. Acquired causes of polymicrogyria include intrauterine infections, particularly cytomegalovirus, intrauterine ischemia, metabolic diseases such as maple syrup urine disease, and peroxisomal disorders such as Zellweger's syndrome (Harding and Pilz 2004).

Macroscopically, the affected area of cortex has a cobblestone appearance. On sectioning the cortical ribbon is thickened in the affected areas (Fig. 26.16). Microscopically the thickened cortex is revealed to consist of multiple thin layers of abnormally folded cortical ribbon (Fig. 26.17). The fused ribbons are separated by an acellular zone within which prominent blood vessels may be seen. Two main histological patterns are seen within the cortical ribbon. The commonest is the unlayered pattern in which the gray matter shows little evidence of a laminar architecture. This is thought to be a disorder of neuronal migration, and the onset is thought to be no later than 16 to 20 weeks of gestation. A less common form is four-layer polymicrogyria with an outer molecular layer and two underlying neuronal layers that sandwich a layer of fibers. This is thought to represent a postmigrational polymicrogyria, and is more commonly associated with acquired injuries such as ischemia and infection.

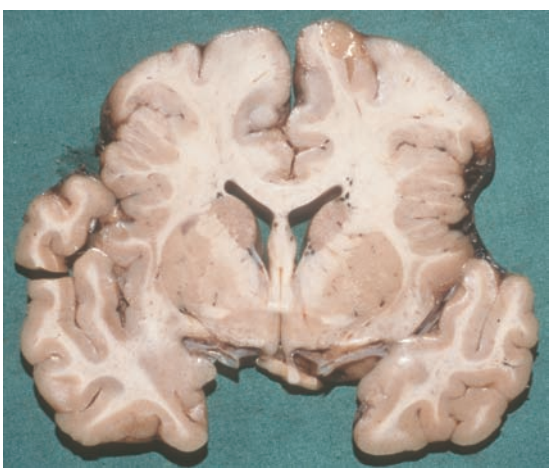

Figure 26.16. A microcephalic brain with areas of polymicrogyria. The cortical ribbon is thickened, particularly over the superior and medial aspects, due to fused folds of cortex.

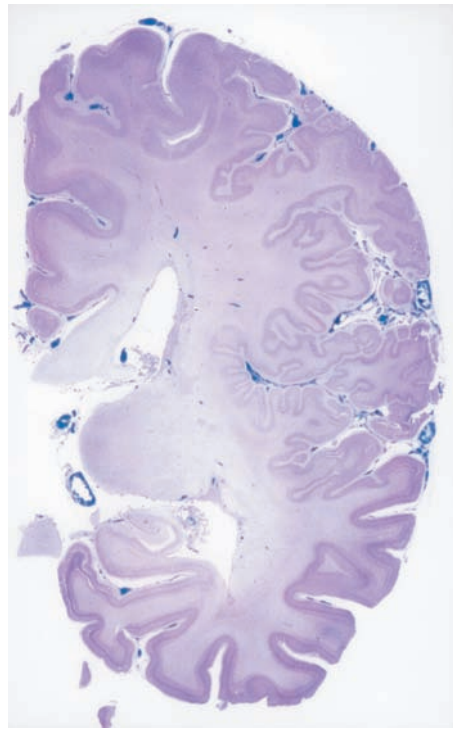

Figure 26.17. Polymicrogyria. Coronal section of cerebral hemisphere with extensive polymicrogyria covering a large area of the lateral surface and extending into the sylvian fissure.

Disorders of Cerebellar, Hindbrain, and Spinal Cord Development

Cerebellar Development

The cerebellum begins to develop at a very early stage (approximately week 3), developing from the rhombic lips, bulging into the developing fourth ventricle, and extending dorsally. These bulges meet in the midline and fuse in a rostral to caudal fashion, the vermis being formed in the midline and the hemispheres developing from the lateral elements. Most neurons of the cerebellum

migrate from the ventricular germinal matrix, although granule cells migrate from the rhombic lip. Cell specification within the hindbrain is controlled by the expression of genes confined to compartments, a process that is increasingly being recognized throughout the developing nervous system (Kiecker and Lumsden 2005). Microscopically Purkinje cells are discernible by approximately 28 to 30 weeks' gestation. Before this stage the Purkinje cell precursors are separated from the granule cells by a clear space, the lamina dissecans. This lamina disappears as Purkinje cells differentiate. The external granule cell layer is well developed at term.

A range of genes have been described in cerebellar development in animal models, including *Otx2, Shh,* and *Pax 2/5* (Wang and Zoghbi 2001).

The main malformations associated with cerebellar development are Chiari and Dandy-Walker malformations. In addition, cerebellar heterotopia and dysplasia are described. Other less common malformations of the hindbrain have been reviewed elsewhere (Parisi and Dobyns 2003).

Chiari Malformations

Chiari malformations refer to the displacement of cerebellar tissue through the foramen magnum. Currently three types are described (Golden 2004c). Type I malformations show the cerebellar tonsils extending into the cervical canal; type II malformations show displacement of the cerebellar vermis and are associated with brainstem abnormalities; type III malformations show displacement of cerebellar tissue into a cervical or low occipital encephalocele. With regard to fetal and neonatal pathology, Chiari type II malformations are of greatest importance, as pregnancy may be terminated when an extensive myelomeningocele is detected. Type I malformations rarely present at this age, and type III malformations are exceptionally rare.

More than 95% of cases of lumbosacral myelomeningoceles have associated Chiari type II malformations, and therefore it is important to look for this malformation in all cases of NTDs. Macroscopically the cerebellar vermis extends over the dorsal aspect of the upper cervical cord (Fig. 26.18). This is best demonstrated in situ at the time of autopsy; demonstration of a vermal malformation can be difficult in an isolated 16- to 20-week-gestation brain. The brainstem shows a range of abnormalities including elongation of the pons and medulla with associated distortion of the fourth ventricle. The inferior colliculi may fuse to produce a characteristic beak-like deformity. Hydrocephalus is commonly seen, although the underlying cause is often not apparent. Polymicrogyria is a not uncommon finding in this setting.

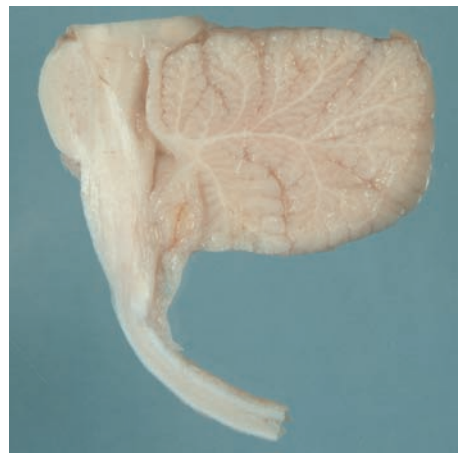

Figure 26.18. Chiari type II malformation with downward displacement of the cerebellar vermis and medulla. A myelomeningocele was present in this case.

The embryological basis of the Chiari type II malformation is currently uncertain, although several theories have been presented. The posterior fossa is always small, and one theory relates to an abnormality of the mesenchyme forming the posterior fossa, the cerebellar abnormality being a consequence of the physical constraints of the small space. Other theories include primary defects in neurulation. However, the theory that is currently receiving the most attention suggests that the cerebellar abnormality is a consequence of abnormal CSF dynamics due to the myelomeningocele. Fetal surgery in utero to repair the myelomeningocele has been documented to resolve an established Chiari type II malformation (Walsh et al. 2001).

Dandy-Walker Malformation

The Dandy-Walker malformation is defined as a cystic dilatation of the fourth ventricle with associated abnormality of the cerebellar vermis. The posterior fossa is also enlarged by the cystic mass. Hydrocephalus is rare in the neonatal period, although is present in virtually all cases by the first year of life.

Dandy-Walker malformations are nearly always sporadic and occur in approximately 1 in 5000 liveborn infants (Parisi and Dobyns 2003). The embryological basis is thought to involve abnormalities in the formation of the choroid plexus within the roof of the fourth ventricle with obstruction of exit foramina, resulting in accumulation of CSF within the fourth ventricle and ballooning of this structure.

It is well recognized that there may be disparity between the prenatal ultrasound diagnosis of a Dandy-Walker malformation and the final autopsy diagnosis after termination. In one study only six of 14 cases (43%) with a prenatal diagnosis of a Dandy-Walker malformation had the same abnormality at autopsy (Carroll et al. 2000). Other conditions that may mimic Dandy-Walker malformations on ultrasound include arachnoid cysts, an enlarged cisterna magna, and Joubert syndrome (see below).

At autopsy the striking feature is cystic dilatation of the fourth ventricle. The vermis may be absent but more frequently there is a residual section superiorly that merges inferiorly with the roof of the fourth ventricle. The cyst results in upward elevation of the tentorium cerebelli and associated sinuses. When the cyst roof is removed, the walls are formed by the cerebellar hemispheres and the floor is the dorsal aspect of the brainstem (Fig. 26.19). Histology of the cyst wall shows a thin layer of leptomeningeal tissue and disorganized glial tissue with an ependymal lining.

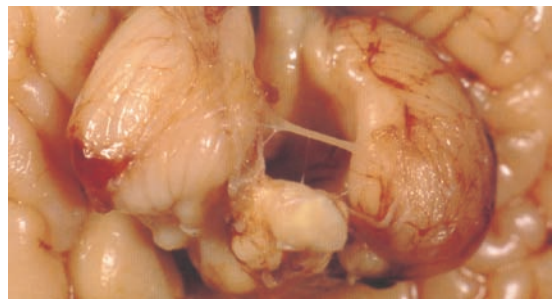

Figure 26.19. Posterior view of the hindbrain from a case of Dandy-Walker malformation. There is an absence of posterior vermis. A remnant of the cyst wall bridges the dilated fourth ventricle.

Rare Cerebellar Dysplasias

Joubert syndrome is a differential diagnosis when Dandy-Walker malformations are being considered. This condition is characterized in infancy by cerebellar and brainstem dysfunction, including ataxia, respiratory problems, and eye movement disorders. The underlying pathology is agenesis of the vermis with associated neuronal heterotopias in the cerebellar hemispheres.

Rhombencephalosynapsis also has vermal aplasia or hypoplasia as the underlying pathological feature, but unlike Dandy-Walker malformations and Joubert syndrome, the cerebellar hemispheres are fused (Friede 1989c).

Brainstem Disorders

Abnormalities of the brainstem are usually associated with more extensive pathology throughout the nervous system, such as olivary heterotopia, which may be seen in neuronal migration disorders and malformations of the cerebellum. Möbius syndrome is defined clinically with facial diplegia and variable cranial nerve involvement. Aplasia or hypoplasia of cranial nerve nuclei have been reported in some cases, while others have been described with focal cranial nerve nuclei necrosis and calcification.

Spinal Cord Disorders

The most common spinal cord abnormalities are the NTDs that are described above. Less common disorders include diplomyelia (cord duplication), diastomatomyelia (cord splitting), and long tract aplasia. Diplomyelia and diastomatomyelia are usually incidental findings at autopsy. A classification has been suggested that describes either single or split dural sacs surrounding the cord sections (Pang et al. 1992). Aplasia of the descending corticospinal pathway has been described,

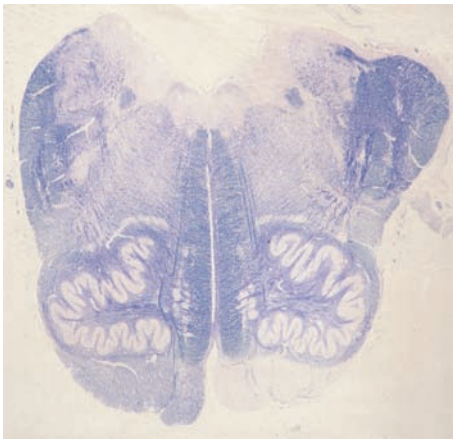

Figure 26.20. Aplasia of the pyramid on one side only, an incidental finding at autopsy. There was no evidence of a tissue reaction to suggest this lesion was acquired.

although again as an incidental finding (Fig. 26.20).

Disorders of the Vascular System

All vascular malformations are rare in infancy. Arteriovenous malformations (AVMs) may present in infancy with intracranial hemorrhage, but up to 50% present with congestive cardiac failure. These have been described as pial AVMs by some authors.

Vein of Galen Aneurysm

The vein of Galen (great cerebral vein) drains blood from the cerebral hemispheres into the dural sinus system. Its developmental precursor, the median prosencephalic vein, may have abnormal connections with intracranial arteries, producing aneurysmal dilatation of the vein. Therefore, despite the commonly applied term vein of Galen aneurysm, this lesion is in fact an arteriovenous malformation (Volpe 2001a). It accounts for up to 30% of pediatric vascular malformations. The typical clinical presentation in neonates is high-output congestive cardiac failure. In infants presentation is usually with hydrocephalus. At autopsy the vascular malformation is obvious. It usually communicates with the posterior cerebral arteries.

Proliferative Vasculopathy and Hydranencephaly (Fowler Syndrome)

This rare condition may be seen as a cause of intrauterine death. Hydranencephaly is described above. The cerebral hemispheres are cystic (Encha-Razavi 2004). Microscopically the developing cortex and white matter are greatly thinned, and glomeruloid vascular structures are seen throughout the brain (Fig. 26.21).

Meningocerebral Dysplasia and Renal Agenesis

Again this is a rare condition that may underlie intrauterine death. The leptomeninges are vascular, and microscopically ectatic vessels are seen throughout the nervous system including the leptomeninges.

Hydrocephalus

Clinically hydrocephalus is a pathological excess of CSF within the ventricular system or subarachnoid space. In simple terms hydrocephalus can be due to the following:

Excess production of CSF
Obstruction to the flow of CSF
Reduced reabsorption of CSF

Loss of brain tissue, such as may be seen after a cerebral infarct, results in an increased volume of CSF and is strictly, therefore, hydrocephalus. This

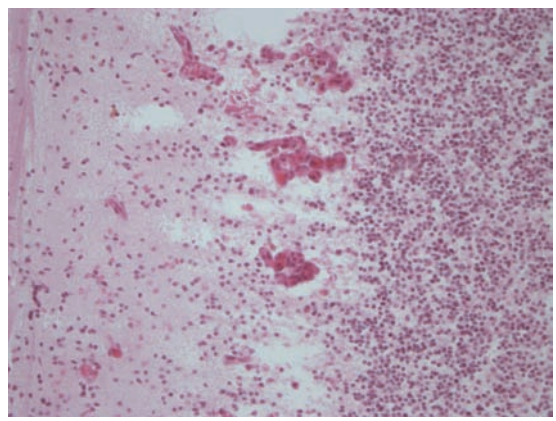

Figure 26.21. Proliferative vasculopathy and hydranencephaly showing glomeruloid vascular proliferations in developing gray and white matter.

Figure 26.22. Parasagittal section of brain with hydrocephalus associated with a large choroid plexus papilloma situated in one lateral ventricle.

loss, however, will not produce symptoms directly related to the increased CSF volume and as such will not be discussed further. In fetal and neonatal practice, the vast majority of cases are due to obstruction of flow, with only rare examples of excess CSF production [such as from a choroids plexus papilloma (Fig. 26.22) or reduced reabsorption (congenital absence of arachnoid villi). This section, therefore, focuses on obstruction to CSF flow.

Hydrocephalus that develops in utero is most commonly a consequence of a malformation or secondary to hemorrhage. In neonates and infants, hemorrhages are still a cause, but infections and tumors increase in frequency.

Diagnosis of fetal hydrocephalus is dependent on intrauterine ultrasound examination. Head growth increases with ventricular enlargement after about 32 to 34 weeks (Volpe 2001b). Prior to this time, ventricular enlargement tends not to be associated with head enlargement. Most cases are associated with malformations, with CNS or extracranial anomalies being found in approximately 80% of cases. Neonatal hydrocephalus can be diagnosed by head enlargement and fullness of the anterior fontanelle.

At autopsy assessment of the hydrocephalic brain can be difficult. Due to the high incidence of associated malformations in fetal hydrocephalus, careful examination of the posterior fossa is required. The brain is often very soft, and disruption of the convexities is commonly encountered. It is important to examine the CSF pathway carefully, and in particular to undertake detailed microscopic examination of the aqueduct and exit foramina related to the fourth ventricle. With careful technique a diagnosis can be achieved in approximately 80% of cases.

The macroscopic appearance of a hydrocephalic brain is similar in most cases. The gyri tend to be flattened with stretching of surface vessels. The white matter is greatly thinned and may be represented by only a thin rim (Fig. 26.23). Although the cortex is also thinned if the hydrocephalus has developed after cortical development, then cortical lamination is usually unaffected. Histologically gliosis is usually apparent. Breaks are seen in the ependymal lining; this is not uncommon in infant brains but when associated with hydrocephalus gliosis is present in the adjacent white matter.

Obstruction at the Foramen of Munro

Very rare developmental malformations at the foramen of Munro have been described, resulting in hydrocephalus (Rekate 2004). However, obstruction at this site is usually acquired secondary to either ventriculitis or tumors such as choroid plexus carcinomas or teratomas (colloid cysts are usually seen in an older age group).

Obstruction at the Aqueduct

Obstruction of the aqueduct is the single most common cause of fetal and neonatal hydrocephalus.

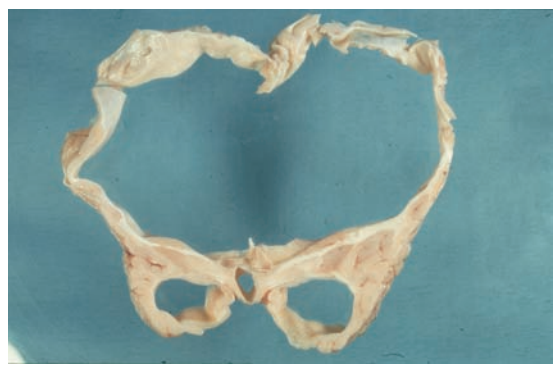

Figure 26.23. A coronal section of a hydrocephalic brain. The cortical ribbon and white matter are thinned, with the white matter changes being most pronounced.

Obstruction of the aqueduct due to maldevelopment of the aqueduct is the second commonest cause of in utero hydrocephalus, second only to a Chiari malformation. Congenital stenosis of the aqueduct of Sylvius (CSAS) is an X-linked recessive condition and is the commonest of the inherited forms of hydrocephalus. These cases usually present in utero, but the hydrocephalus may become arrested and present after birth. Thumb contractures have been described in many affected fetuses and should be sought in all cases of fetal hydrocephalus. The abnormality has been linked to the *LICAM* gene at Xq28 (Rosenthal et al. 1992).

Acquired causes of obstruction at the aqueduct are usually secondary to gliosis or hemorrhage (Friede 1989d). In all cases of fetal and neonatal hydrocephalus it is important to examine the midbrain microscopically. The aqueduct may be completely absent, or may show a branching pattern (Fig. 26.24). In acquired obstruction gliosis can be demonstrated by immunohistochemistry.

Obstruction of the Exit Foramina

Obstruction of the exit foramina may be produced by malformations, such as Dandy-Walker or severe Chiari malformations, or may be acquired, with infection and hemorrhage being the most frequent pathologies. Malformations should be apparent at the time of autopsy. Acquired conditions can usually be demonstrated by serial sectioning through the fourth ventricle. The leptomeninges may be thickened, and hemosiderin-containing macrophages may be apparent.

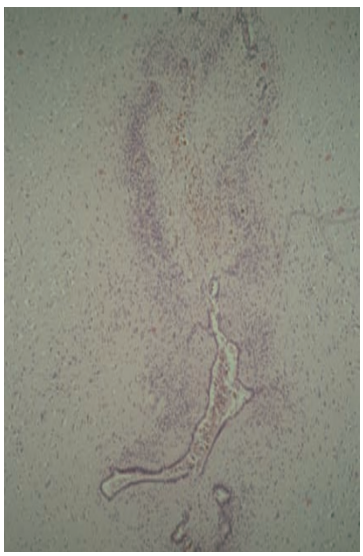

Figure 26.24. A case of aqueductal stenosis secondary to previous hemorrhage and gliosis.

Obstruction of the Basal Cisterns

Occlusion of the basal cisterns prevents the flow of CSF. The commonest causes of adhesion formation in the basal cisterns are infections and hemorrhages. Hemorrhages may be seen after intraventricular hemorrhages, and hydrocephalus is a common and serious complication in long-term survivors of intraventricular hemorrhages, and is particularly seen in prematurity.

Examination of Treated Hydrocephalic Brains

Insertion of a shunt can produce dramatic changes with restoration of virtually normal parenchymal volume from a scarcely visible rim in initial scans. Examination of the brain after shunting can be difficult due to the combined effects of the initial pathology and of treatment. Meningitis and hemorrhage cause dense thickening of the meninges, which may be firmly adherent and orange or yellow if blood pigment persists. There may be evidence of old subdural hematoma and occasionally parenchymal loss or hemorrhage at the site of the shunt placement. The white matter is usually atrophic and gliosed and the corpus callosum thin. The collapsed ventricles may be distorted or slit-like, and if intraventricular pressure has been very low, adhesions may develop between the walls of the collapsed ventricles. Occasionally, there is upward herniation of the cerebellar hemispheres through the tentorium.

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27
Acquired Diseases of the Nervous System

Colin Smith and Marian V. Squier

Acquired disorders of the nervous system are responsible for considerable perinatal mortality and for a devastating chronic handicap in children. Identification of acquired lesions and their distinction from malformations can be difficult because damage to the brain in the early weeks of pregnancy disrupts the orderly and complex sequence of development and leads to structural abnormality. It is important to recognize that brain development is a continuum that begins at conception and continues into the third decade, and that damage can occur at any time in this period.

This chapter describes the more common acquired conditions likely to be encountered in routine postmortem examination of infants and fetuses. Some knowledge of the normal development of the brain is important in understanding these conditions, and excellent accounts are available in the work of Larroche (1977a) and in an anatomical atlas, *Development of the Human Fetal Brain* (Feess-Higgins and Larroche 1987).

Pathological Reactions in the Human Fetal Brain

Pathological cellular reactions proceed at different rates in the immature and adult brains. The importance of recognizing reactive changes is twofold. First, in some circumstances, they enable the pathologist to time an injury. Second, they cause patterns of damage, for example, gliosis, cyst formation, and calcification, that persist and may be identified many years later on brain scans or on pathological examination of tissue. The times shown in Table 27.1 are derived from personal cases and from the literature.

Edema

The immature brain swells rapidly. Due to the considerable space around the brain and unfused skull sutures, brain swelling rarely causes herniation in infants. In the fixed brain the key features of edema are prominence of the cortical ribbon and compression of ventricles and sulci.

Cell Death

Cell death may take one of two main forms. *Necrosis* is passive cell death. When metabolism ceases, the cell membrane breaks down and the cell becomes brightly eosinophilic (Fig. 27.1). The nuclear membrane disintegrates with lysis of nuclear chromatin.

Apoptosis is a form of active cell death mediated by activation of a cascade of intracellular enzyme reactions terminating in breakdown of DNA. Apoptosis is the mechanism of programmed cell death that assists in regulation of cell numbers in brain development. The morphological characteristics of apoptosis include rounding and shrinkage of the nucleus with intense, nuclear basophilia (pyknosis). The nucleus breaks up into a number of rounded particles (karyorrhexis) (Fig. 27.2).

Gliosis

There is a mistaken but widespread belief that gliosis is a feature of later developmental stages.

Table 27.1. Approximate timing of cellular responses to injury in the fetal and neonatal brain

Macrophages	
Microglial proliferation	3 hours–3 days
Macrophages	4–5 days
Macrophages showing evidence of phagocytosis	4–6 days
Astrocytes	
Astrocyte proliferation	12 hours–4 days
Astrocytes with cytoplasmic processes	3–11 days
Astrocyte fibrillary gliosis	6 days
Capillaries	
Endothelial swelling	1–3 days
Endothelial reduplication	5 days
Coagulation necrosis	3 hours
Retraction balls	3 hours
Neuronal karyorrhexis	12–48 hours
Mineralization	8–14 days
Cysts	14–42 days

Source: Banker and Larroche (1972); Sherwood et al. (1978); Roessmann and Gambetti (1986); Armstrong et al. (1987); Darrow et al. (1988); Ellis et al. (1988); Lou (1989).

Astrocytes are identified from 7 weeks in cultures of human brain (Elder and Major 1988) and at 15 weeks' gestation by immunocytochemistry (Roessmann and Gambetti 1986). Damage occurring as early as 18 weeks promotes a vigorous astrocyte and macrophage response (Squier et al. 2000) (Fig. 27.3).

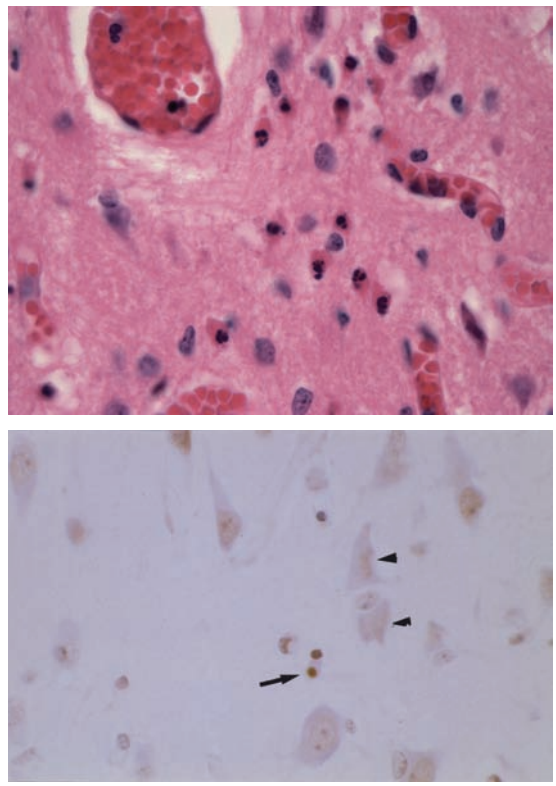


Figure 27.2. (A) Apoptotic bodies are numerous in the ventral pons after in utero ischemia. The nuclei of the damaged cells are fragmented producing apoptotic bodies (H&E). (B) The terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) technique labels DNA fragmentation in apoptosis. A labeled apoptotic cell is marked (arrow). Necrotic cells (arrowheads) remain pale.

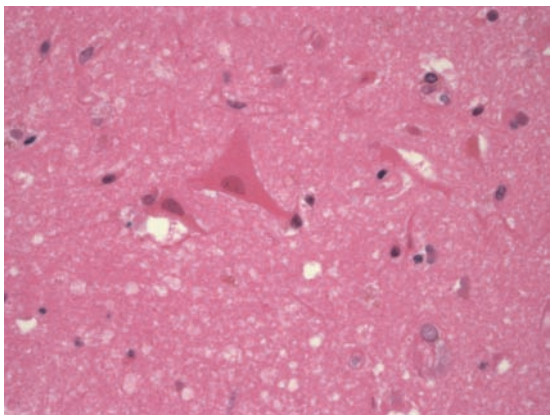


Figure 27.1. In neonates, cortical ischemia produces a characteristic response in mature neurons. The neurons show nuclear shrinkage and marked cytoplasmic eosinophilia [hematoxylin and eosin (H&E)].

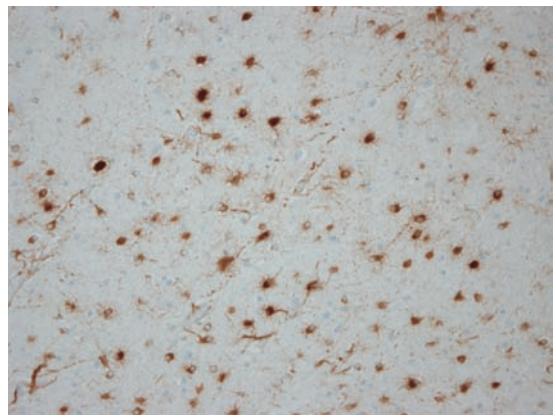


Figure 27.3. Gliosis, highlighted by glial fibrillary acidic protein (GFAP) immunohistochemistry. The reactive astrocytes are hypertrophied with abundant cytoplasm and numerous processes.

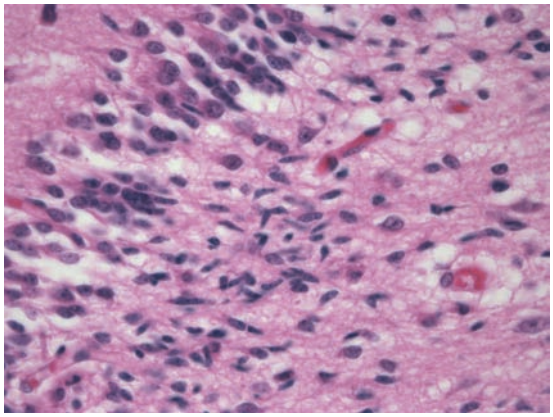


Figure 27.4. Dentate gyrus of the hippocampus showing an intense microglial reaction on the deep border of the granule cell layer (H&E).

Phagocytosis

The earliest response is seen in microglia, the brain's intrinsic tissue population of phagocytes. Microglia are characterized by their elongated, almost rectangular nuclei. A pronounced microglial response in the inner border of the dentate gyrus of the hippocampus (Fig. 27.4) is considered a reliable marker of hypoxic–ischemic insult in infants less than 9 months of age (Del Bigio and Becker 1994). Macrophages ingest debris and contribute to cyst formation. They take up and break down red cells and may persist for years after injury as a lasting record of previous hemorrhage (Fig. 27.5).

Capillary Proliferation

In the first days after injury, capillary endothelial cells become thicker, and the nuclei, instead of being thin, become rounded or oval and plump in their linear profiles. Capillary branching and proliferation are seen 5 to 8 days after injury. Capillary proliferation is identified in magnetic resonance imaging (MRI) scans as a bright signal first seen 5 to 6 days after injury (Fig. 27.6).

Mineralization

This occurs readily in the fetal brain and is first seen within 1 week of injury. It appears as basophilic granules with macrophages, nerve cells, and processes. It persists and is seen on computed tomography (CT) scans in later life. Mineralization is a hallmark of viral infections when it tends to be widespread throughout the brain and may be particularly dense in the periventricular tissues. Following ischemic damage it is seen in areas of old infarction and in the thalamus (Fig. 27.7).

Patterns of Injury

The pattern of acquired damage often suggests the etiology. Infectious agents show a predilection for particular parts of the brain, and ischemic lesions may reflect vascular territories and watershed zones. The developing nervous system shows changing patterns of susceptibility to hypoxic and ischemic injury. Ischemic damage to the white matter is characteristic of the preterm infant when

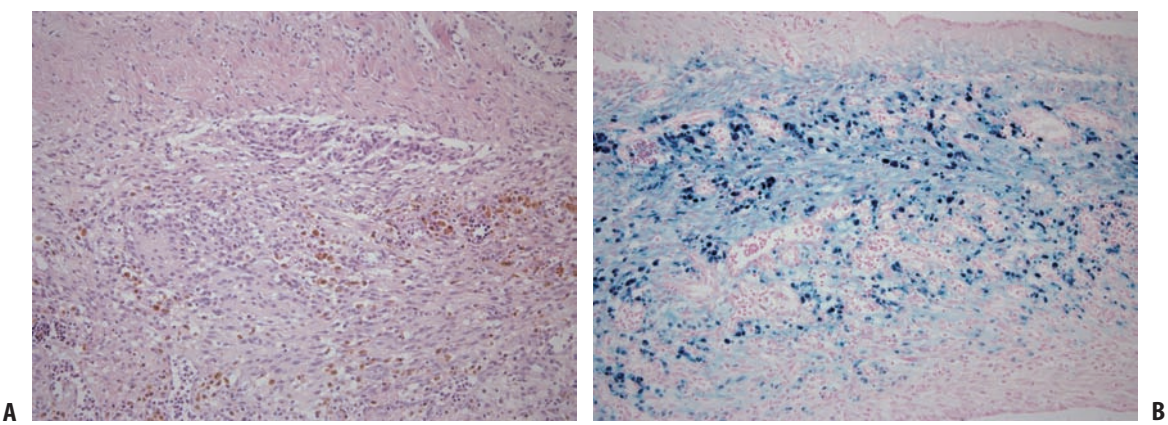


Figure 27.5. (A) An organizing subdural membrane that contains numerous golden-brown hemosiderin containing macrophages (H&E). (B) An iron stain highlights hemosiderin containing macrophages (Perl's). The iron pigment is stained blue.

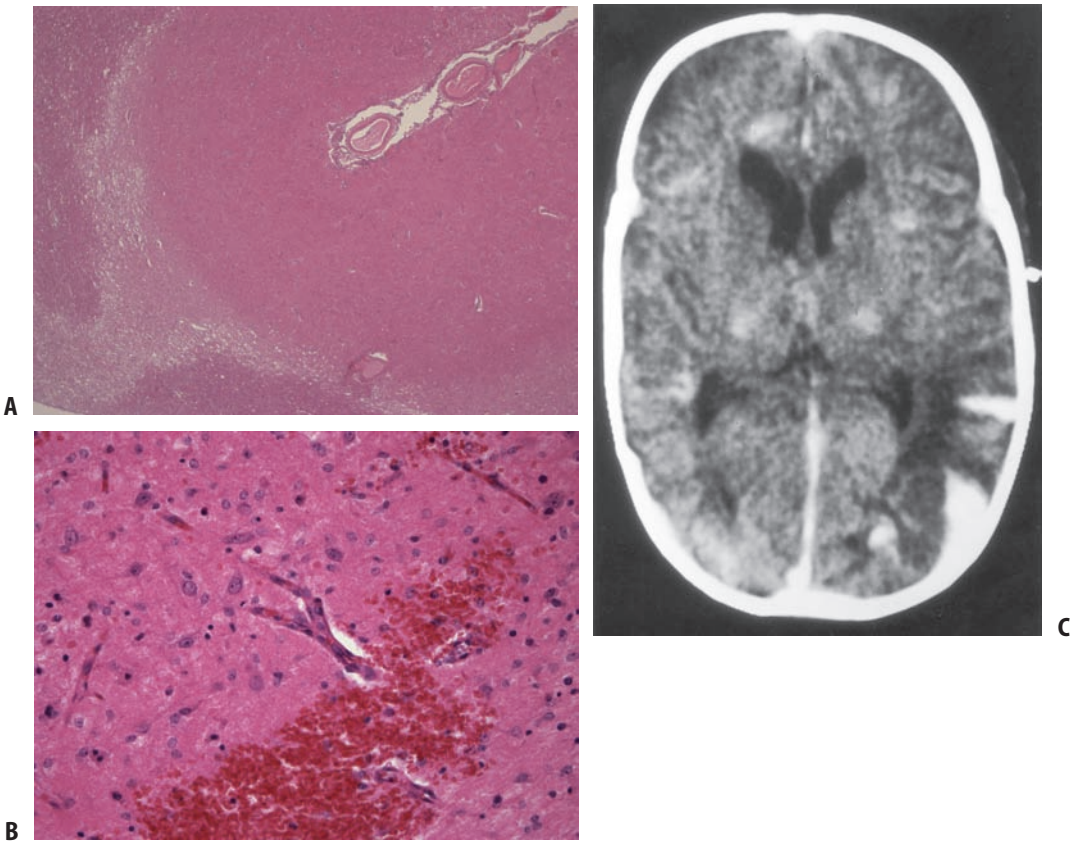


Figure 27.6. (A) Cerebral cortex after ischemia. The entire cortex is infarcted, and separation between the cortex and underlying white matter can be seen as an area of pallor and vacuolation. (B) Capillaries adjacent to an area of infarction showing endothelial swelling (H&E). (C) Magnetic resonance imaging (MRI) scan of the brain of an infant 9 days after global ischemia. Bright signals in the cortex are known as "cortical highlighting."

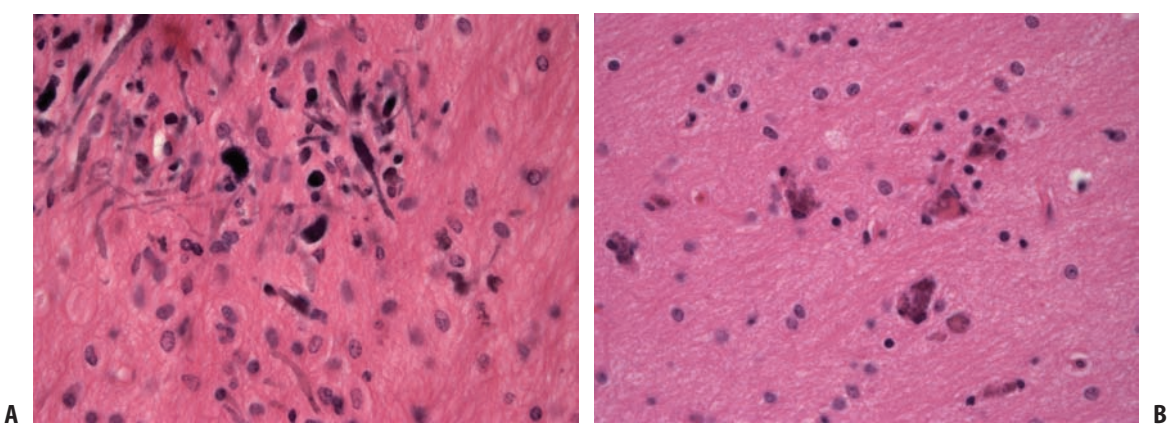


Figure 27.7. (A) Mineralization of axonal profiles from a region of periventricular leukomalacia (H&E). (B) Neuronal mineralization in the thalamus from a case of cerebral palsy. The child died age 3 years (H&E).

the gray matter may escape unharmed, while at term, the gray matter usually bears the brunt of injury. Although these patterns of damage may provide clues to timing and nature of injury, the elapse of time between damage and death complicates the final picture due to secondary degenerative processes. So, for example, reduction in cerebral white matter may result from loss of cortical neurons, while the dentate and brainstem nuclei may degenerate following damage to the cerebellar cortex.

Although hypoxia–ischemia is undoubtedly a common cause of injury during development, there are many other causes of prenatal brain damage (Blair 1996). There is increasing evidence that cerebral damage is associated with maternal infection, placental inflammation, and funisitis, mediated by the action of cytokines (Dammann and Leviton 1997; Yoon et al. 1997; Rothwell 1999; Wu and Colford 2000). Metabolic disorders such as impaired iodine metabolism are also associated with developmental brain abnormalities (Pharoah and Connolly 1995).

Clinical, pathological, and epidemiological studies show that brain damage commonly occurs in the antenatal period and that the majority of severely handicapped children have no history of birth difficulties (Becher et al. 2004, 2006). Many babies showing signs of asphyxia at birth may have been suffering for a considerable time in utero before the onset of labor or delivery. The importance of careful scrutiny of clinical notes together with detailed macroscopic and histological examination of the brains of stillborn infants and early neonatal deaths cannot be overemphasized. Only in this way can we hope to prevent brain damage by establishing the timing and causes of acquired lesions. Association of placental inflammatory and vascular lesions with developmental lesions and intraventricular hemorrhage (Grafe 1994; Hansen et al. 1998) has reinforced the importance of careful macroscopic and histological examination of the placenta (see Chapter 3). This evidence has been increasingly demanded in litigation.

Hemorrhage

The site of intracranial hemorrhage is to a large extent determined by the age of the infant and the cause of bleeding. The two most important causes are hypoxia and trauma; deep hemorrhages tend to result from hypoxia and occur in premature infants, while superficial hemorrhages into the brain, the meninges, the scalp, and the skull are usually traumatic and affect term infants. These are dealt with in Chapter 13. Rare causes are coagulopathies, infections, vascular malformations, tumors, and iatrogenic causes such extracorporeal membrane oxygenation (ECMO). Up to 3.5% of healthy term babies have evidence of intracranial hemorrhage without an identifiable cause (Heibel et al. 1993).

Subdural Hemorrhage

Subdural hemorrhage (SDH) may be seen in babies in relation to various obstetric factors. It results from deformation of the head during delivery and risk factors include the duration of engagement of the head and mechanism of delivery. Bleeding may develop in the infratentorial subdural space secondary to laceration of the tentorium cerebelli and associated sinus and vein damage (including the vein of Galen), or due to traumatic separation of the squamous and lateral parts of the occipital bone damaging the occipital sinus. In the supratentorial subdural space bleeding may be a consequence of damage to bridging veins producing convexity bleeding or, rarely, due to falx laceration resulting in predominantly interhemispheric bleeding. The degree of bleeding is variable but in some cases can be massive and fatal. However, many subdural bleeds in the neonatal period are likely to be clinically insignificant. An MRI-based study designed to identify SDHs (Whitby et al. 2004) found the highest risk for asymptomatic SDH was the use of forceps after an attempted ventouse delivery. In asymptomatic cases the SDH had completely resolved, as assessed by MRI, by 4 weeks. Modern obstetrics practice has reduced the incidence of birth-related SDH (Luerssen 1991).

Blood clot in the subdural space is organized by invading fibroblasts and capillaries. Microscopic examination of a SDH may give some indication of the age of the lesion, although timings are inaccurate. The appearance of hemosiderin-containing macrophages at about day 3 in the evolution can be helpful in aging and is best demonstrated by a Perl's stain (Fig. 27.5). The altered blood persists as a bright orange or yellow stain for months or years.

In young children SDH is most commonly seen in relation to a head injury, although a number of alternative causes need to be considered particularly in possible nonaccidental injury (NAI) cases (Kemp 2002).

Subarachnoid Hemorrhage

Subarachnoid hemorrhage (SAH) is a frequent finding in neonatal autopsies, particularly in premature infants, and is probably common in neonates with no obvious clinical syndrome. However, SAH is rarely clinically significant. It may be primary or secondary, usually as a consequence of the extension of an intraventricular hemorrhage through the ventricular system (Fig. 27.8). The mechanism of primary SAH development is poorly understood but may be related to birth trauma or circulatory events related to prematurity. The very rare occurrence of massive SAH in infants is related to severe perinatal hypoxia. Subarachnoid hemorrhage as a result of ruptured cerebral aneurysms are described in infancy but are rare (Grode et al. 1978).

Subependymal Germinal Matrix and Intraventricular Hemorrhage

Subependymal germinal matrix hemorrhage (SEH) and intraventricular hemorrhage (IVH) characteristically occur in the premature infant. The incidence of SEH is directly related to the gestational age at the time of birth. It is seen in 40% to 50% of infants born at less than 26 weeks, but are present in fewer than 5% of infants born at 32 weeks or longer. In addition, birth at less than 28 weeks is associated with more extensive bleeding (Berger et al. 1997). Several obstetric antecedents have interesting associations with development of IVH. There is a strong association with placental inflammation (Hansen et al. 1998), while pregnancy-induced hypertension (PIH) is associated with a reduced risk of IVH. It is not clear whether this is due to PIH itself or its treatment (Paneth et al. 1994; Perlman et al. 1997). The onset is usually between 12 and 72 hours after birth (Wigglesworth 1989), and susceptible infants are usually sick with respiratory distress syndrome or its complications.

The germinal matrix is a transient, embryonic structure consisting of a layer of densely packed immature cells found just beneath the ependymal lining of the lateral ventricles. A prominent collection is found close to the head of the caudate nucleus. The matrix is the site of active cell generation; it has a dense network of thin capillary channels lined by endothelium, but lacking muscle or collagen in their walls. During the first half of gestation neuronal precursors develop and migrate out into the cerebral cortex. Later, glial cells are produced before the germinal matrix begins to involute, and by 36 weeks only small groups of immature cells remain in the subependymal zone and clustered around vessels of the deep white matter.

Pathology of Germinal Matrix Hemorrhage

The macroscopic appearance of fresh hemorrhage in the fixed brain is of a mass of deep red blood clot of uniform appearance (Fig. 27.9). The hem-

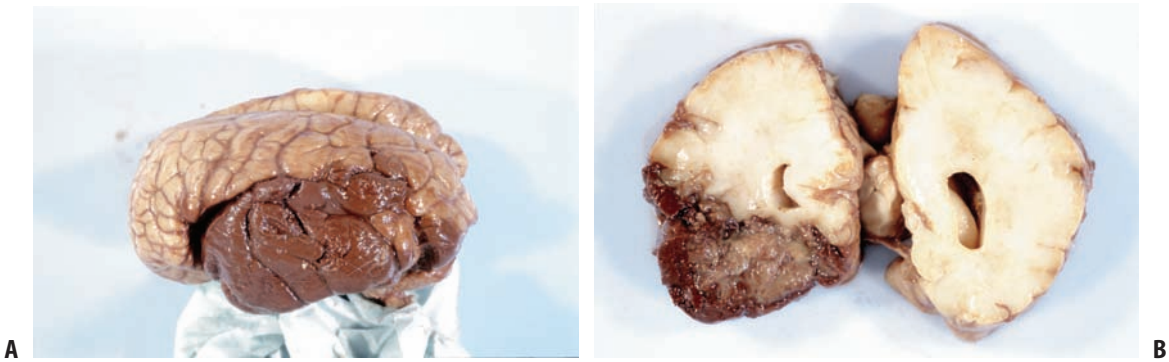


Figure 27.8. (A) Subarachnoid hemorrhage overlying the temporal lobe in a preterm infant. (B) Coronal section from the same brain extensive hemorrhagic infarction underlying the region of subarachnoid hemorrhage.

orrhages may be unilateral or bilateral, and often extend into the ventricular system. Their localization tends to be related to age; hemorrhage originates more frequently over the body of the caudate nucleus before 28 weeks of gestation, and further forward over the head of the caudate nucleus, close to the level of the foramen of Monro after 29 weeks (Hambleton and Wigglesworth 1976; de Reuck 1984).

The breach in the ependymal lining may not be obvious even in cases in which there is abundant intraventricular hemorrhage. After some weeks the hemorrhage becomes paler in color and begins to undergo lysis. In long-term survival the only evidence of previous hemorrhage may be a small residual, smooth walled subependymal cyst.

Histology of fresh SEH shows disruption of the matrix by blood cells. The capillary walls show evidence of ischemic damage in cases examined soon after bleeding (Marin-Padilla 1995). Polymorph reaction is not seen. After 2 to 3 days large macrophages with red cells within their cytoplasm are seen in the region of the hemorrhage (Sherwood et al. 1978; Darrow et al. 1988).

During fixation blood pigments diffuse out of the clot into adjacent parenchyma and on macroscopic examination may give the impression of parenchymal involvement. Close inspection is usually sufficient to demonstrate integrity of the parenchyma, but histological examination may be required to be certain.

Intraventricular extension results from rupture of the ependyma, and blood clot is found in the lateral ventricles, often extending through the third ventricle (Fig. 27.10), the midbrain aqueduct of Sylvius, and the fourth ventricle. Escape of blood through the exit foramina of the fourth ventricle allows blood to collect around the brainstem and cerebellum, presenting the characteristic features on the base of the uncut brain (Fig. 27.11). Altered blood pigments cause brown discoloration of the entire ventricular system and meninges at the base of the brain.

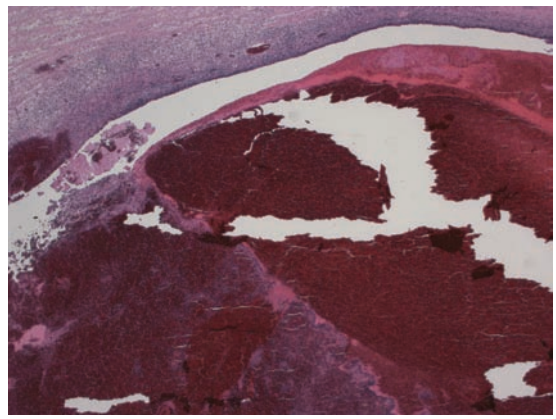
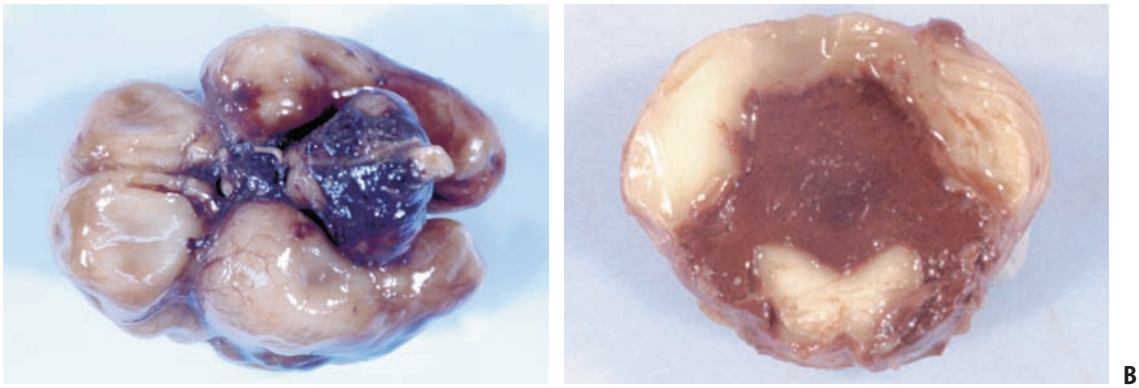


Figure 27.10. Section of brain showing a germinal matrix hemorrhage that ruptures through the ependymal lining and into the ventricular system. This hemorrhage also extended into the surrounding white matter. Early neonatal death of a preterm infant (H&E).

The presence of blood in the ventricular system prompts a response in the ependymal lining and underlying tissues. Proliferating glial cells grow through and over the ependyma and form a thick lining. Fragments of ependyma persist as rosettes or isolated strips within this thick band of glial tissue. Groups of macrophages containing altered blood cells are found on the surface and trapped within the proliferated glial tissue. Macrophages containing iron pigment are still identifiable years later.

This ependymal and subependymal reaction is of significance when it occurs either in the cerebral aqueduct or in the outflow foramina draining the fourth ventricle or in the arachnoid villi close to the sagittal sinus where cerebrospinal fluid (CSF) is resorbed. In these sites it can obstruct the flow of CSF, causing hydrocephalus. When the aqueduct alone is obstructed the lateral and third ventricles dilate, while obstruction of the outflow from the fourth ventricle causes the fourth

Figure 27.9. Bilateral germinal matrix hemorrhages with extension into the ventricular system.



A B

Figure 27.11. (A) Intraventricular hemorrhage. Characteristic appearance of subarachnoid blood clot around the base of the brain of a 24-week-gestation fetus. (B) Coronal slice through the hindbrain shows blood clot distending the fourth ventricle and escaping through the lateral outflow foramina to fill the subarachnoid space around the medulla.

ventricle and cerebral aqueduct to dilate as well. Obstruction to CSF drainage at the arachnoid villi causes communicating hydrocephalus where fluid is increased over the brain surface as well as within the ventricular system.

Pathogenesis of Germinal Matrix Hemorrhage

The walls of the immature blood vessels of the germinal matrix are physically weak, and vulnerable to sudden hemodynamic changes. In common with other cerebral vessels they have large numbers of mitochondria (Oldendorf et al. 1977), implying a high metabolic rate and susceptibility to oxygen deprivation.

Autoregulation of cerebral blood flow, which protects the capillary networks from large fluctuations in systemic pressure, is disturbed in the sick infant (Pryds and Edwards 1996). The cerebral circulation then becomes susceptible to sudden changes in systemic blood pressure (Lou et al. 1979; Milligan 1980) such as those that may occur during routine medical or nursing procedures (Volpe 2001a). In addition, there is increased vulnerability to free radical injury (Ment et al. 1985), and increased fibrinolytic activity has been described in the germinal matrix (Gilles et al. 1971).

Associated Lesions

Up to 75% of babies with SEH have damage to other parts of the brain. The lesions most frequently associated with SEH are periventricular leukomalacia and pontosubicular necrosis (Skullerud and Westre 1986; Armstrong et al. 1987). The most serious associated brain lesion is parenchymal hemorrhagic infarction.

Parenchymal Hemorrhagic Infarction

Approximately 15% of neonates with SEH show extension of the hemorrhage into surrounding parenchyma with a characteristic periventricular lesion. The incidence of these periventricular hemorrhages increases with lower gestational age (Golden et al. 1997). In contrast to periventricular leukomalacia, it is usually unilateral. In long-term survivors there is asymmetrical ventricular dilatation. Microscopic examination shows clusters of perivascular hemorrhages along the course of the

veins fanning out into the centrum semiovale (Gould et al. 1987), suggesting venous infarction. Positron emission tomography has demonstrated reduced blood flow to an extensive area of the hemisphere around a matrix hemorrhage, which may precede, and persist for some time after, the bleed (Lou 1989).

Sequelae of Subependymal Germinal Matrix and Intraventricular Hemorrhage

The clinical syndrome seen is dependent on the severity of the hemorrhage, and long-term outcome is a reflection of the associated parenchymal damage. Several grading systems exist based on cranial ultrasound. One of the earliest and most frequently used is that described by Papile (1978). This system entails four grades of SEH: grade 1, confined to the germinal matrix; grade 2, with extension into the lateral ventricle but no ventricular dilatation; grade 3, with distention of the lateral ventricle; and grade 4, the same as grade 3 but with associated hemorrhage into adjacent brain parenchyma.

The early consequences include hemorrhagic infarction of adjacent parenchyma, when fibers from the motor cortex may be damaged in their course to the internal capsule, and acute hydrocephalus may occur due to obstruction of CSF flow by blood clot. Hydrocephalus may also be a late complication due to chronic reactive changes to blood in the ventricular system.

Neurological Sequelae

Small (grade 1) hemorrhages are encountered frequently, and have an excellent prognosis (Lou 1989). More than 50% of infants who have large hemorrhages are normal on follow-up (de Vries et al. 1985; Volpe 2001b). The loss of germinal matrix cells, which are a source of future glial cells, some of which will be responsible for myelin synthesis, does not appear to jeopardize future myelination (Johnson 1987; Van de Bor et al. 1989). The prognosis is much worse when there is associated parenchymal damage (Leviton and Paneth 1990). The main problems seen are motor problems, particularly spastic hemiparesis with equal involvement of both upper and lower limbs, and cognitive problems.

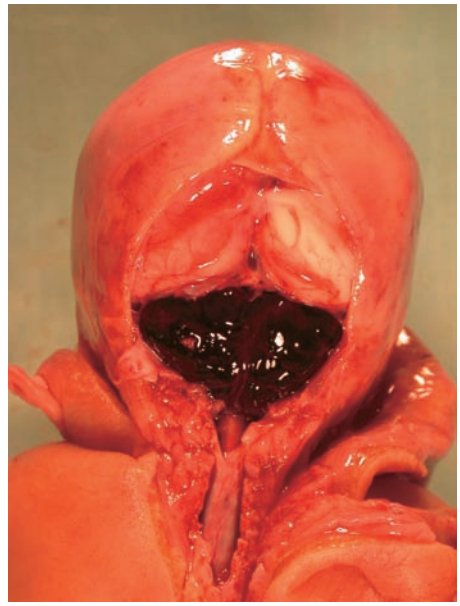


Figure 27.12. Intracerebellar hemorrhage with extensive bihemispheric damage. Neonatal death of a 26-week preterm with pseudomonas septicemia.

Intracerebellar Hemorrhages

Cerebellar hemorrhages are common in preterm neonates, being seen in 21% of cases from one study (Martin et al. 1976). In addition, autopsy examination has been shown to identify many intracerebellar hemorrhages not identified by CT imaging (Flodmark et al. 1980). They are rare in term neonates.

Cerebellar hemorrhages are usually, but not always, multifocal. They are typically small in size, although these become confluent to form a large blood clot filling most of a single hemisphere, or both hemispheres (Fig. 27.12). The surrounding cerebellar tissue is friable.

Acquired Parenchymal Damage

Brain Damage Due to Hypoxia and Ischemia

This is probably the commonest type of acquired brain damage in the fetal and neonatal brain. The pattern of injury is to some extent age dependent but also dependent on the nature, intensity, and duration of the insult. Many other factors may contribute to the extent of damage, including

carbon dioxide retention, acidosis, circulatory arrest, hypotension, and hypoglycemia.

A diagnosis of hypoxic–ischemic damage is sometimes made by exclusion. This is worrying in view of mounting epidemiological evidence suggesting that other conditions such as maternal infections, abdominal trauma, metabolic disorders including iodine deficiency, and mercury intoxication may cause chronic brain damage during development, which result in clinical syndromes of cerebral palsy indistinguishable from those due to presumed hypoxic–ischemic injury (Blair 1996).

Clinical Manifestations of Hypoxic–Ischemic Injury

Infants who have recently suffered severe asphyxia are extremely ill with coma, seizures, hypotonia, poor respiratory effort, and various other neurological signs. The clinical term used to describe this syndrome is *hypoxic-ischemic encephalopathy,* which is usually clinically graded as an aid to assessing prognosis; 35% have a history of adverse intrapartum events, 35% have additional predisposing antepartum events, and in 20% there is evidence that antepartum events alone are responsible (Volpe 2001c).

The chronic outcome is usually some form of *cerebral palsy*. This term covers a wide range of neurological impairment resulting from nonprogressive damage to the developing brain spanning minor learning disorders as well as severe mental and motor impairment.

Timing of Parenchymal Damage

In 1861, Little described a series of children with spastic diplegia and attributed their condition to difficult birth. Since then, cerebral palsy has often been considered the result of birth injury. However, epidemiological studies over the last few years have shown that in only about 10% of cases of cerebral palsy is there a history of birth asphyxia, and prenatal factors are more important as predictors of cerebral palsy (Blair 1996). In 1897, Freud pointed out that birth usually is not harmful, and birth difficulties may be symptomatic of preexisting adverse conditions in utero. Indeed, asphyxia has been identified before birth in growth-restricted fetuses (Soothill et al. 1987). Brain damage of antenatal origin has been demonstrated by ultrasound and CT scans (Gunn et al. 1985; Larroche 1986; Stoddard et al. 1988; Tenoris et al. 1988) and by neuropathological study of the brains of stillborn infants and very early neonatal deaths. An incidence of up to 44% has been reported (Sims et al. 1985; Roland et al. 1987; Ellis et al. 1988; Squier and Keeling 1991). It is obviously very important to identify damage of prenatal origin and distinguish it from damage arising during labor and delivery not only to further our understanding of their causes and prevention but also for medicolegal reasons.

Identifying the precise time and nature of damage is difficult, particularly in the antenatal period. There are few cases with well-documented histories of specific insults in utero. When the brain is examined within days or weeks of an insult, it may be possible to time the insult by the nature of the cellular reactive changes, but these cases are infrequent.

Certain patterns of damage correspond to injury occurring in specific periods of gestation.

Damage in the First Trimester

Damage in the first trimester leads either to disruptive defects or to fetal loss. Identification of damage to specific structures within their period of formation enables the precise timing of an injury.

Damage in the Second Trimester

Injuries during this period of gestation can interrupt the process of neuronal migration, which continues until 20 weeks. This results in failure of migration, neuronal heterotopia, and cortical malformation. Polymicrogyria is seen in damage before gyration has occurred, that is up to 28 weeks (Barth 1987).

Complete infarction, for example following destruction of a major cerebral artery, results in cystic breakdown and development of porencephalic cysts; polymicrogyric cortex on the borders of these cysts is evidence of their origin before 28 weeks (Williams et al. 1976).

Injury in the second trimester has been illustrated in a small number of cases of amniocentesis at 16 to 19 weeks in which needle penetration of

the fetal brain has resulted in focal tissue loss and gliosis, as well as heterotopias and cortical disruption at the site of injury (Squier et al. 2000).

Damage in the Third Trimester

White Matter

Between 28 and 36 weeks' gestation, the white matter is particularly vulnerable to damage, although this may also occur up to and even after term. The particular vulnerability of the white matter has been ascribed to a tenuous blood supply derived from the terminal branches of end arteries that arise on the brain surface and pass through the entire depth of the developing brain wall, but detailed anatomical studies have shown an extensive vascular network in the developing white matter (Kuban and Gilles 1985). Blood flow to the white matter of the normal preterm infant, however, is very low (Borch and Greisen 1998). During the first half of the last trimester, myelination is beginning in the deep white matter. The area is metabolically highly active, as oligodendrocytes migrate in it to begin myelin synthesis, and so it is very vulnerable to a reduction in oxygen supply (Volpe 1989).

Gilles et al. (1983) suggested that white matter damage may result from bacterial endotoxemia. An association with infection that does not directly invade the fetal brain has been shown by pathological (Sims et al. 1985; Bejar et al. 1988) and epidemiological studies, suggesting that white matter damage is mediated by cytokine activity (Leviton and Paneth 1990; Wu and Colford 2000).

White matter damage takes several forms that are related both to the time of insult and to its nature.

Periventricular Leukomalacia

Coronal slices of the fixed brain show the well-developed focal necroses as white or yellow spots, several millimeters in diameter, and sometimes centrally cavitated, in the deep white matter (Fig. 27.13). The most common areas of involvement are close to the frontal horn of the lateral ventricle and in the occipital white matter. The deep white matter elsewhere usually has a light brown discoloration and radial vessels appear very prominent.

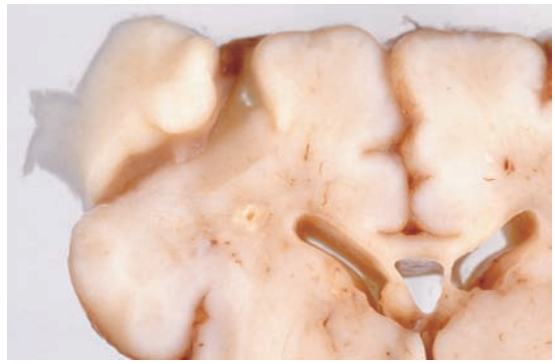


Figure 27.13. Periventricular leukomalacia. Fixed brain slice showing the chalky white spots of white matter damage lying adjacent to the angles of the lateral ventricles.

Histological appearance depends on the age of the lesions. In the early stages an irregular zone of coagulation necrosis is seen (Fig. 27.14A). Nuclear pyknosis and microglial reaction occur early, and then astrocytic proliferation and collections of macrophages are seen (Fig. 27.14B). Retraction balls may be prominent in adjacent tissue. These are rounded eosinophilic masses, the swollen proximal ends of severed axons. Immunocytochemistry using β-amyloid precursor protein (β-APP) stains these swollen axons (Bell et al. 2005). Capillary endothelial reactions around the zone of necrosis may be very striking. The endothelial nuclei become plump and rounded, and the endothelium thickens and may even be reduplicated. Nuclear karyorrhexis is common in capillary endothelium (Squier and Keeling 1991). In older lesions a peripheral zone of deeply basophilic sticks or balls are seen, the remains of mineralized cells and axon stumps or capillaries (see Fig. 27.7A). This accounts for the yellow or white discoloration of the macroscopic lesions.

Away from the focal necroses are more diffuse changes in the white matter. Early on, the white matter is edematous and may contain clusters of retraction balls. There is diffuse infiltration by macrophages and reactive astrocytes. Capillary endothelial thickening is prominent, and karyorrhectic nuclei are seen in capillary endothelium and in scattered cells throughout the white matter. In the later stages the white matter is diffusely gliotic.

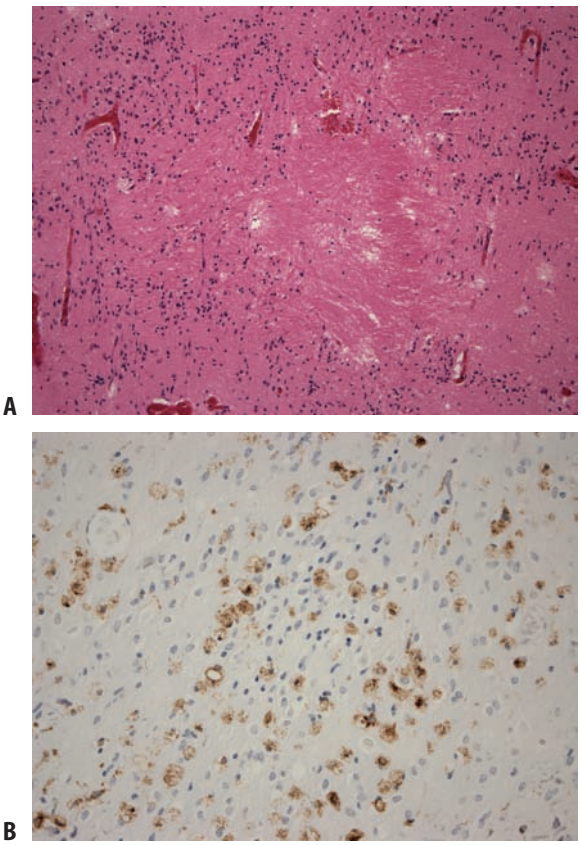


FIGURE 27.14. (A) Periventricular leukomalacia. A low-power micrograph showing an area of infarction in the periventricular deep white matter (H&E). (B) Macrophage infiltration begins within a few days of white matter damage and can be highlighted by immunohistochemistry (CD68 immunohistochemistry).

The pathophysiology of periventricular leukomalacia is now well defined (Blumenthal 2004; Folkerth 2006). The premature brain has poorly developed penetrating vessels, resulting in a watershed region within the periventricular white matter (Takashima and Tanaka 1978). This area is therefore susceptible to alterations in blood flow, setting up a cycle of ischemia and reperfusion injury. The oligodendrocyte precursor cells are thought to be sensitive to free radical injury, free radicals being generated by ischemia, and excitotoxicity. Microglial activation may cause further tissue injury (Folkerth 2006). In addition, axonal integrity is disrupted by the ischemic damage.

Diffuse White Matter Ischemia (Telencephalic Leukomalacia)

Diffuse white matter ischemic change has been reported up to 40% of infants postmortem (Gilles et al. 1983; Squier and Keeling 1991).

The macroscopic changes may be subtle and easily confused with postmortem artifact. The deep white matter is gray or light brown, and radial vessels are prominent. Yellow streaks may be seen around or adjacent to blood vessels. The histological changes are much as those seen in the white matter of infants with periventricular leukomalacia away from the focal necroses (Fig. 27.15). It is found throughout the cerebral white matter, but most consistently in the occipital lobes. Examination of gray matter shows it to be largely spared, but scattered karyorrhectic nuclei may be found particularly in the hippocampus, brainstem, and cerebellar cortex.

The presence of large numbers of reactive astrocytes alone is not sufficient to constitute pathological change in the white matter, as these cells may be difficult to distinguish from the normal glia of myelination (Norman 1978, Rorke 1982a). Astrocytosis may occur in a number of metabolic diseases.

Multicystic Leukoencephalopathy

This term describes replacement of most of the cerebral white matter by multiple large cysts

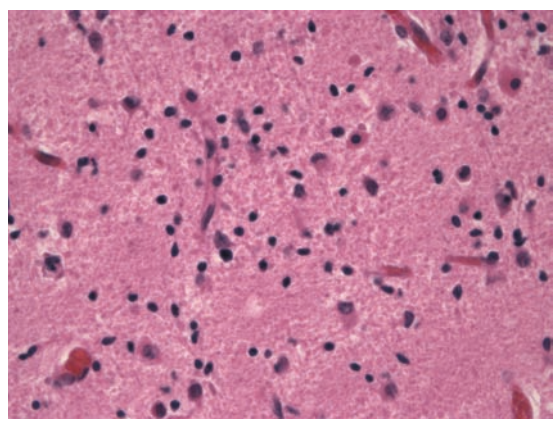


FIGURE 27.15. Diffuse white matter ischemic damage. Endothelial nuclei are prominent and plump. There are reactive astrocytes and infiltration of the tissue by macrophages (H&E).

with smooth, clean walls. In some cases the cysts are found predominantly in the subcortical white matter, but in others they occupy almost all of the white matter extending to the edge of the lateral ventricles, which are dilated due to tissue loss (Fig. 27.16). The cortex is a thin band stretched over the cysts, and deep gray matter and brainstem are white and very firm to touch.

Histology shows extensive fibrillary gliosis in the walls of the cysts with very little myelination in the cerebral hemispheres. The overlying cortex is almost totally devoid of nerve cells and replaced by glial cells and small cystic cavities packed with macrophages that persist for many years.

Several cases of multicystic leukomalacia are documented in the literature. They all occurred between 30 and 44 weeks of gestation and usually resulted from an incident causing an abrupt interference in fetal blood supply including maternal hypotension due to anaphylaxis (Erasmus et al. 1982), maternal involvement in a motor vehicle accident, or maternal butane intoxication (Larroche 1986).

Ultrasound scans can differentiate between periventricular and subcortical cysts in surviving infants. There is a much poorer neurological outcome in infants with subcortical cysts or a mixed pattern than in those with purely periventricular cysts (De Vries et al. 1987, 1989).

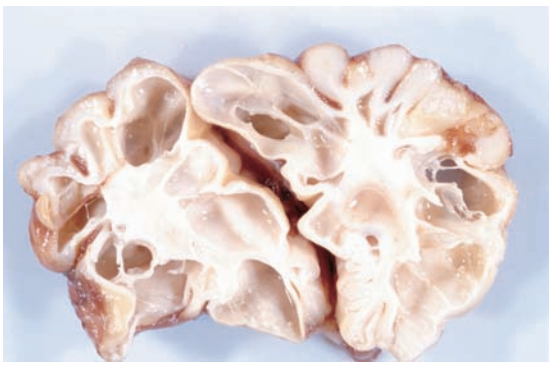


Figure 27.16. Multicystic encephalopathy. Large cysts are seen throughout both cerebral hemispheres. There is relative preservation of periventricular white matter.

Clinical Correlations of White Matter Damage

The increased use of clinical scanning studies, such as ultrasound, CT, and MRI, has led to great advances in our understanding of the long-term evolution of white matter damage and its clinical consequences.

Early periventricular infarction shows as bright echogenic flares on ultrasound scans. These may fade or progress to cyst formation. The cysts may later collapse and disappear with dilation of the lateral ventricles due to tissue atrophy. In some cases the cysts fuse with the lateral ventricles, causing dilation with irregular or asymmetrical walls. Clinical follow-up of children with cystic lesions shows a very high proportion to have a serious neurological handicap including spastic quadriplegia, spastic diplegia, impairments of speech and vision, and developmental delay. The MRI studies show delays in myelination following periventricular leukomalacia (Johnson et al. 1987, Van de Bor et al. 1989).

Gray Matter

Gray matter damage may be seen at any stage of gestation, its pattern depending on the nature and severity of insult. Although gray matter damage is thought to be characteristic of the term brain, infarction of the basal ganglia is described in fetuses of 24 and 28 weeks' gestation (Cohen and Roessman 1994). The cortex is more typically vulnerable at term (Larroche 1977b; Volpe 2001d).

Thalamus and Basal Ganglia

At term the thalamus and lentiform nucleus are particularly vulnerable. Sequential MRI scans of infants who have severe hypoxic–ischemic encephalopathy at term show vulnerability of the thalamus and posterior putamen (Rutherford 1996) (Fig. 27.17).

In the early stages, macroscopic examination shows pink or brown discoloration of these nuclei. Histological examination shows nuclear karyorrhexis, necrosis, glial and macrophage infiltration, and intense capillary proliferation from 5 days after injury. Some neuronal profiles become calcified. The chronic effects are either cyst formation

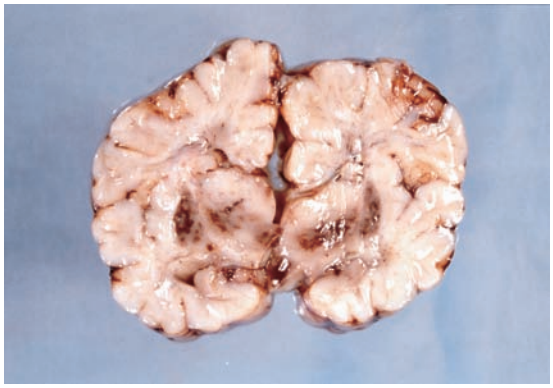


Figure 27.17. Hypoxic-ischemic encephalopathy. Coronal slice of a fixed infant brain showing recent infarction of the basal ganglia and thalamus.

or atrophy with dense gliosis and persistence of calcified neurones.

Status Marmoratus

The "marbled state" of the deep nuclei (Fig. 27.18) is seen after 6 to 9 months of life and is recognized on MRI scans. The appearance is due to aberrant myelination of glial fibrils. It occurs in infants who have had basal ganglia damage with neuronal loss and gliosis at term, and does not develop until 6 months when myelination occurs in the deep nuclei (Friede 1989a).

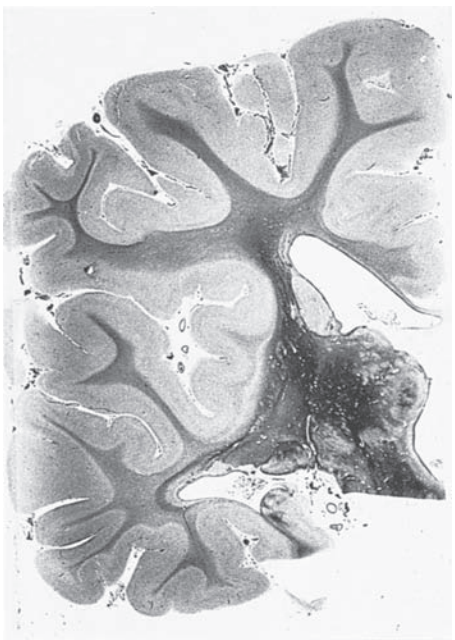


Figure 27.18. Status marmoratus. Section of cerebral hemisphere of a child of 6 months with cerebral palsy. The thalamus shows abnormal, patchy myelination.

Cerebral Cortex

Damage to the cerebral cortex, including the hippocampus, is characteristic of the term infant. The distribution of damage is not uniform but tends to involve parasagittal watershed areas (Volpe 2001e), the hippocampus, and the subiculum.

There is further regional vulnerability within the cortex itself; the depth of the sulci tend to be involved more frequently than crests of gyri. This results in shrinkage of the deep cortex of sulci, leading to a "mushroom" appearance of the gyri known as *ulegyria*. This is characteristic of cortical injury in the term brain and may be recognized on later brain scans (Fig. 27.19).

After 5 or 6 days capillary proliferation is prominent, and large capillaries are seen dipping in from overlying meninges. This is identified on CT and MRI scans as cortical highlighting (see Fig. 27.3).

Later, neurons are lost and replaced by dense gliotic tissue with shrinkage of the damaged cortex. There is secondary loss of underlying white matter and atrophy of fiber tracts originating in the damaged regions.

Brainstem

The nuclei of the cranial nerves in the brainstem develop very early and are vulnerable to ischemic damage. Brainstem necrosis is described in infants suffering a single severe and acute asphyxial episode and who have a very poor prognosis (Pasternak 1993). It has been termed "cardiac arrest encephalopathy" (Janzer and Friede 1980). It is usually associated with damage elsewhere, particularly in the thalamus. The pattern of damage is striking, there being symmetrical bilateral necrosis of the nuclei of the tegmentum running throughout the entire length of the brainstem (Fig. 27.20).

Less severe or chronic insults to the brainstem cause selective damage to cranial nerve nuclei, which are more vulnerable than other brainstem

structures before 34 weeks (Dambska et al. 1987). This form of intrauterine brainstem injury is one of the causes of Möbius syndrome or congenital facial diplegia.

Figure 27.19. (A) Late effects of ischemic damage. Coronal slice of the brain of a 5-year-old child with cerebral palsy, epilepsy, and blindness. The lateral ventricles are dilated due to loss of tissue, and the corpus callosum is thin. Areas of old cortical damage are seen as thinning of the cortex, which is most marked on the medial surfaces of the hemispheres and in watershed areas. Here examples of ulegyria are seen (arrows) with more severe involvement of the deep sulcal cortex than the crests of gyri. (B) Coronal section of a brain with marked ulegyria stained with the neuronal marker MAP-2. Note persistence of the neurons in the crests of gyri and cystic atrophy of the cortex in the depths of sulci (microtubular associated protein; MAP).

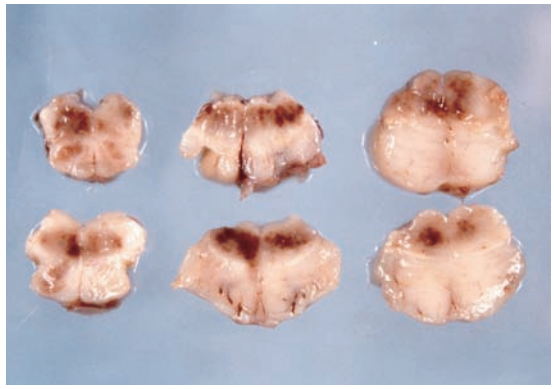


Figure 27.20. Brainstem ischemia. There is recent infarction of brainstem nuclei particularly within the tegmentum.

Pontosubicular Necrosis

This is a well-recognized association of neuronal necrosis involving the pontine nuclei, which are diffusely spread between the fiber bundles of the corticospinal tracts in the base of the pons and neurons of the subiculum, which is adjacent to the hippocampus. The early stages are characterized by large numbers of karyorrhexes; later there is atrophy with gliosis and sometimes cysts filled with macrophages. Skullerud and Westre (1986) found this lesion in 59% of preterm infants either alone or combined with other lesions, but it was encountered less frequently (43%) in a recent Scottish study (Bell et al. 2005). It has also been noted in preterm infants with germinal matrix hemorrhage (Armstrong et al. 1987).

Cerebellum

Several patterns of damage are recorded. Hypoxic damage sometimes results in selective loss of cortical neurons in the cerebellar hemispheres with relative sparing of the midline, phylogenetically older structures (Rorke 1982b).

In some instances damage is manifest by sclerosis occurring only in the depths of the sulci bordering on central white matter (Fig. 27.21). Histological examination shows remarkable propensity for cells of the granule layers to die by apoptosis. Purkinje cells, in contrast, undergo eosinophilic change and necrosis.

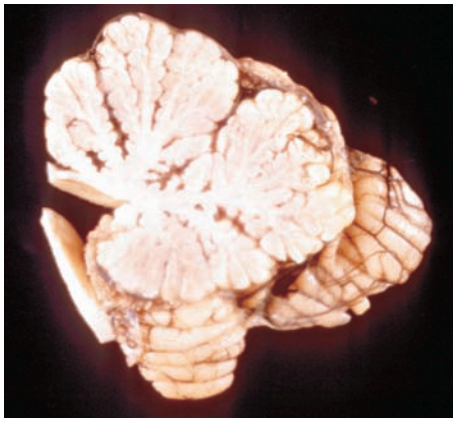


Figure 27.21. Cerebellar ischemia. This cross section shows significant vermal atrophy secondary to a birth related ischemic injury.

Spinal Cord

Removal and examination of the spinal cord is often neglected. However, it is not infrequently the site of ischemic damage (see Fig. 13.29 in Chapter 13), and destruction of anterior horn cells may play a role in the motor deficits observed in infants with hypoxic–ischemic encephalopathy (Clancy et al. 1989). Intrauterine ischemic damage to anterior horn cells has been demonstrated as one cause of arthrogryposis multiplex congenita (Horoupian and Yoon 1988).

Porencephaly

This term is used for large cystic defects of the cerebral hemispheres, resulting from infarction of brain tissue, including both cortex and underlying white matter (Fig. 27.22). The cause may be ischemic or hemorrhagic. Brown discoloration around the cyst wall indicates old hemorrhage, and debris may be found lining the ventricular system. Porencephalic cysts may occur at any time, but onset in the early months of gestation is indicated when malformation or polymicrogy-

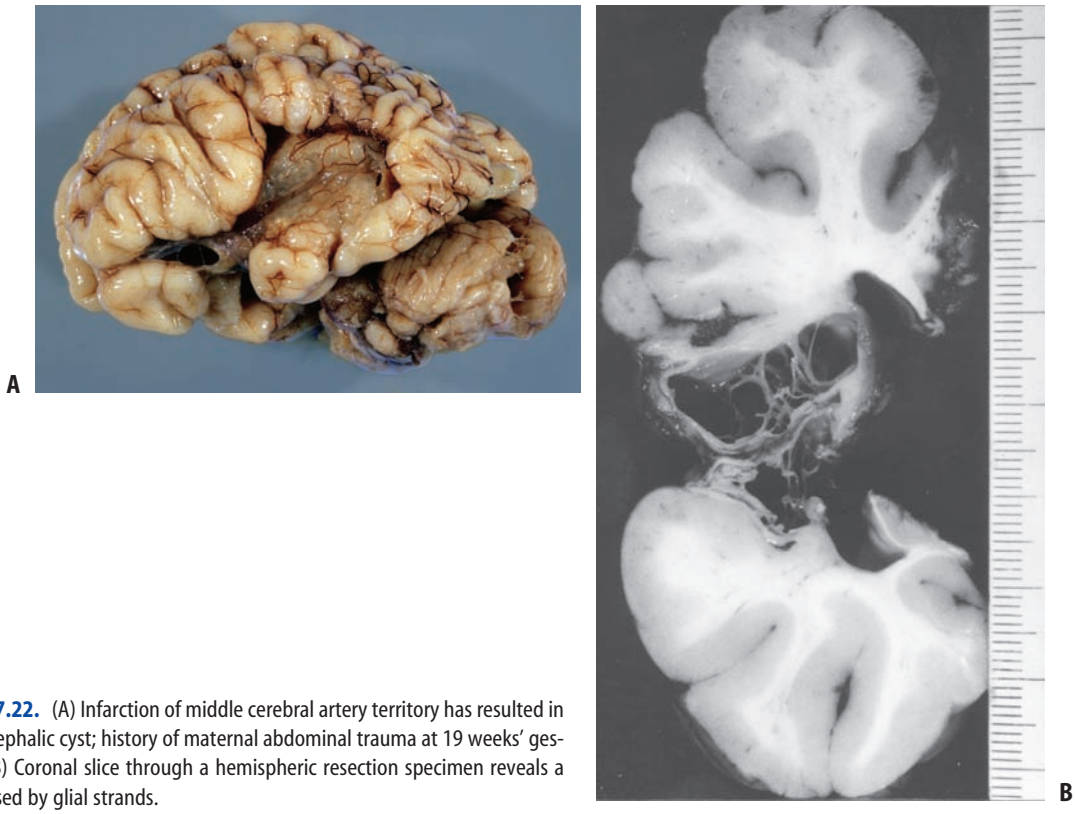


Figure 27.22. (A) Infarction of middle cerebral artery territory has resulted in a porencephalic cyst; history of maternal abdominal trauma at 19 weeks' gestation. (B) Coronal slice through a hemispheric resection specimen reveals a cyst crossed by glial strands.

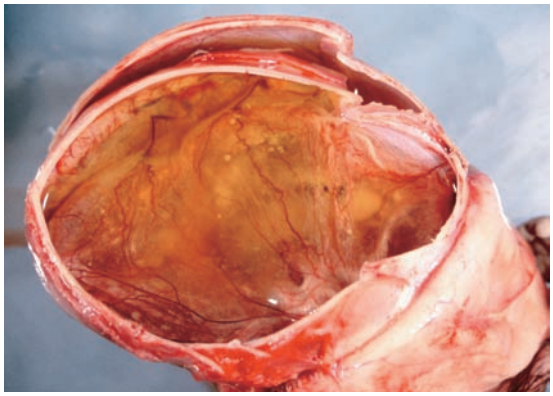


Figure 27.23. Hydranencephaly. The cerebral hemispheres are absent being replaced by a thin fibrous cystic membrane. Nodules of glial and neuronal tissue may be found within this membrane.

ria is seen around the edge of the cyst (Williams et al. 1976).

Hydranencephaly

This term denotes bilateral destruction of the major part of both cerebral hemispheres, which are each replaced by a thin-walled, fluid-filled cyst (Fig. 27.23). The membrane covering the cyst is made up of an outer layer derived from leptomeninges and an inner layer of glial tissue in which occasional neurons and clusters of macrophages, sometimes containing hemosiderin, are seen.

The damage usually involves areas of supply of the middle cerebral arteries or of the internal carotid arteries with sparing of the inferior aspects of the occipital lobes and temporal lobes, basal ganglia, brainstem, and cerebellum. Preservation of a parasagittal strip of cortex has led to use of the term "basket brain."

The history of pregnancy usually reveals no clue as to an etiology for such disastrous brain damage, and many of these infants have normal deliveries with no evidence of neurological abnormality for weeks or even months after birth (Rorke 1982c).

Arterial Occlusions

Fetal and neonatal cerebral infarcts are multifactorial, and risk factors include maternal factors, for example, sepsis, placental dysfunction, diabetes, and drugs, and genetic factors, for example, thrombophilias and rare metabolic disorders such as mitochondrial disorders (Marret et al. 2001). The left middle cerebral artery is most commonly involved.

Examination of the vessels in the early stages may show evidence of arterial thrombosis (Larroche 1977c), but in older cases the vessels are almost invariably normal. Larroche (1977c) describes six infants with infarction localized to middle cerebral artery territory and found arterial thrombosis in three of them. The lesions may occur in pre-, peri- or postnatal life. They are not infrequently demonstrated on neonatal brain scans of infants who are otherwise well and who have a good prognosis. Other infants develop hemiplegia, and in a small proportion severe intractable epilepsy results.

Infections of the Nervous System

Infection remains an important cause of stillbirth, particularly in developing countries (Goldenberg and Thompson 2003). Infection may result in fetal death through several pathways, including maternal sepsis associated with pyrexia, placental inflammation resulting in reduced oxygen perfusion, and direct infection of the fetus, the organism gaining access through the placenta or membranes.

Viral Infections

Brain damage due to viral infections in the developmental period is well recognized. Most features are quite nonspecific, such as microgyria, focal micronodular calcification, and subependymal cysts. The pattern of damage and the effects on subsequent brain development are more dependent on the time of infection than on the specific agent involved. Sensitive techniques are now available to detect viral DNA in tissue sections, including immunocytochemistry, in-situ hybridization, and in-situ solution polymerase chain reaction (Gressens et al. 1994).

Cytomegalovirus

This is the most common viral infection of the immature brain and is identified in 1% of all live

births. No vaccine is available. These infections may be expected to be more frequent in the future in older children, as opportunistic infections as part of acquired immune deficiency syndrome (AIDS) encephalopathy. The virus is usually transferred to the fetus via the placenta during a primary maternal infection, which is often asymptomatic (see Chapter 16). The most damaging infections occur during the first and second trimester.

The brain in the infected neonate is usually small with ventricular dilation due to tissue loss. There is a chronic leptomeningitis with evidence of damage to the underlying cortex (Fig. 27.24A). The brain is often deep brown in color due to increased vascularity, and periventricular calcification stands out as a prominent white band. Flecks of calcification occur throughout the cerebral tissue (Fig. 27.24B). About two thirds of reported cases are microcephalic, and a minority develop hydrocephalus.

Histology shows predominant damage in the walls of the lateral ventricles. Cells containing inclusions may be scattered or in small clusters throughout the tissues with a variable glial response and associated calcification. The characteristic and diagnostic intranuclear inclusions (Fig. 27.24C) are found in a variety of cell types, predominantly endothelial cells and mature neurons and some astrocytes. Polymicrogyria in the cerebral and cerebellar cortex is thought to be secondary to vascular disturbances during early development rather than to a direct cytopathic effect of the virus on developing neurones (Marques Dias et al. 1984).

Rubella

Congenital rubella is now very infrequent in the United Kingdom, as only 20 cases are reported each year, many in immigrants. Intrauterine rubella infection is acquired by transplacental transfer and has a more dramatic effect on the eye and the ear than on the central nervous system itself. Neuropathology in neonates shows focal tissue necrosis and nodules of calcification close to vessel walls. Chronic inflammatory cells may be found in the leptomeninges and walls of the ventricular system. Malformations, such as gyral abnormalities may result from vasculopathy in utero. Microcephaly

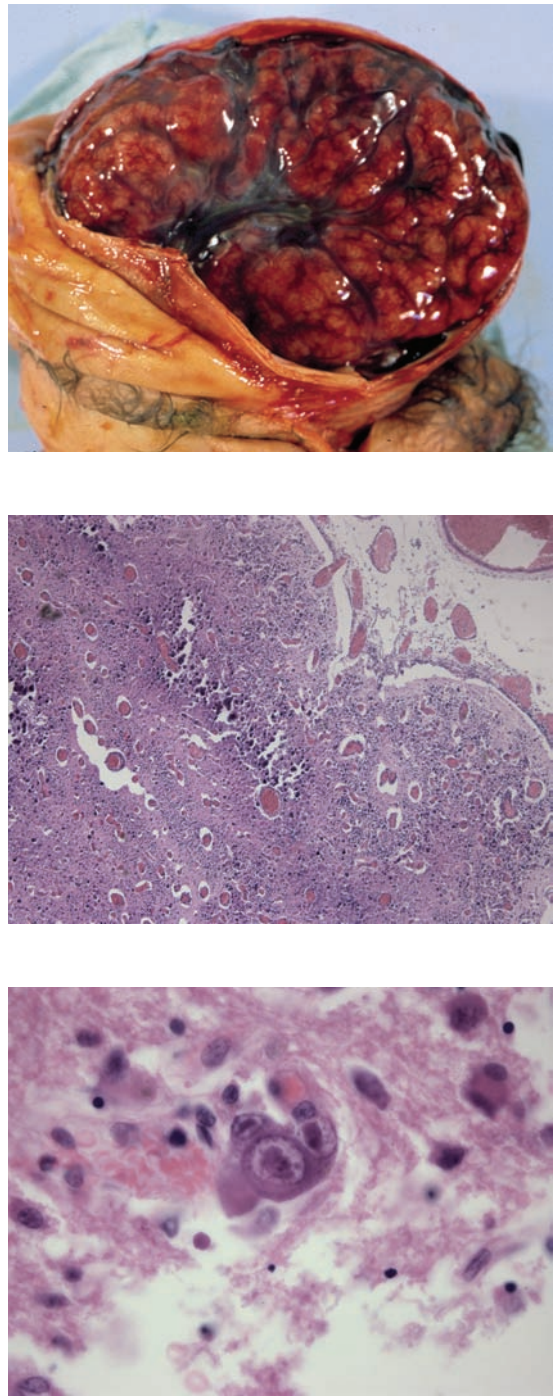


Figure 27.24. (A) Congenital cytomegalovirus (CMV) infection. There is a chronic leptomeningitis with extensive vascularization and opacity. Gyri are narrower than normal. (B) Low power micrograph showing necrosis and mineralization within the cortex (H&E). (C) Characteristic "owl's eye" inclusions can be seen within residual neurons, endothelial cells and glial cells (H&E).

and delayed myelination may be due to impaired cellular replication (Volpe 2001f).

Herpes Simplex

Herpes simplex infection of the brain is rare. It is acquired more commonly at birth or in the neonatal period than by transplacental infection. Type II herpes is the virus usually involved.

There is meningeal infiltration by lymphocytes, mononuclear cells, and granulocytes with discrete proliferation of microglial cells and lymphocytes in the parenchyma. Eosinophilic nuclear inclusions (Cowdry type A) are seen in glial cells, particularly oligodendrocytes, and neurons close to inflammatory foci (Friede 1989c).

Brain damage can be devastating and extend widely through the brain, causing cystic destruction and hemorrhage (multicystic encephalomalacia). In two personally reviewed cases MRI scan appearances were mistaken for severe hypoxic–ischemic damage following nonaccidental injury.

Diagnosis is established by immunocytochemistry or in-situ hybridization. Demonstration of virus in blood vessels, blood cells, and sensory nuclei of the brainstem suggests both blood-borne and sensorineural routes of spread to the brain (Gressens 1994).

The mortality rate in neonatal herpes is high (47%), and survivors of encephalitis have a high incidence of neurological impairment (Malouf and Oates 1995).

Human Immunodeficiency Virus

This virus continues to be a worldwide pandemic involving both adult and pediatric populations. In the United States fetal infection with human immunodeficiency virus (HIV) accounts for more than 90% of infections in children (Volpe 2001g). Up to half of all hospital admissions in Africa are currently for pediatric AIDS.

Neurological manifestations in the neonate are rare, but 80% of cases present clinically by the age of 2 with nonspecific, nonneurological conditions including failure to thrive, lymphadenopathy, and candidiasis (Volpe 2001g). Neurological features are of encephalopathy. Due to increased use of antiretroviral therapy and control of opportunistic infections, survival is increasing, and AIDS-related dementia and other central nervous system (CNS) manifestations will increase in the young adult population. Current evidence suggests that the virus does not cause damage by primary infection of neurons but causes neuronal death by production of neurotoxins from HIV-infected brain macrophages (Gelbard and Epstein 1995).

Transmission of HIV from seropositive mothers to their infants occurs transplacentally, at delivery, and probably also via breast milk (European Collaborative Study 1992). Transmission can be prevented with zidovudine if HIV-positive mothers are identified. By 18 months of age, one third of congenitally infected infants will be HIV positive or have AIDS, and one fifth of this group will have died (Blanche et al. 1989). Although the fetus may be infected in utero, no cerebral manifestations of infection in the fetus have been described; 62 fetuses of seropositive mothers whose pregnancies were terminated showed no cerebral lesions (Mulliez et al. 1989).

The incidence of encephalopathy in HIV-positive children is high. The pathological features are similar to those described in adults: cerebral atrophy, generalized myelin pallor, microglial proliferation sometimes in focal collections "microglial nodules" multinucleate giant cells, calcification of basal ganglia, and gliosis (Sharer et al. 1986).

Protozoal Infections

Toxoplasmosis

Toxoplasmosis is a common opportunist infection in the nervous system in AIDS. Congenital toxoplasmosis usually causes hydrocephalus, although this may not be present at the time of birth. It results from obstruction of CSF pathways due to ependymitis or from massive tissue destruction causing hydrocephalus "ex vacuo." Less commonly tissue destruction results in microcephaly.

The brain shows scattered soft yellow foci, sometimes cystic, up to several centimeters in diameter. The ventricles are dilated and the walls irregular and necrotic. There may be obvious obstruction of the aqueduct by necrotic debris. Lesions involve cerebral hemispheres, brainstem, and spinal cord but the cerebellum is relatively less involved.

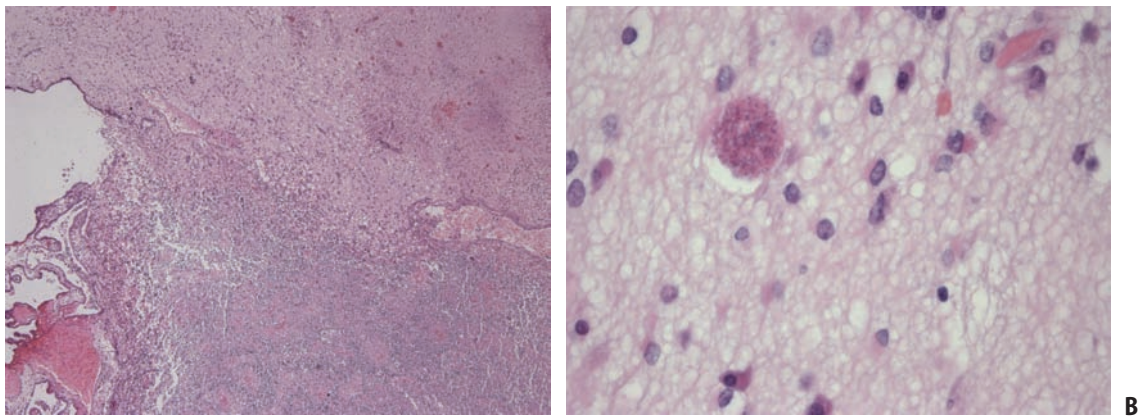


Figure 27.25. (A) Low-power micrograph from a case of congenital toxoplasmosis. There is extensive necrosis within the cerebral hemisphere (H&E). (B) At high power, organisms may be free within the necrotic and adjacent viable tissue, or may be encysted, as illustrated (H&E).

Histology shows foci of necrosis containing macrophages and lymphocytes bordered by capillary and astrocytic proliferation. Calcification is obvious in the form of large granules or a fine basophilic dust. Inflammation and destruction of the ependyma is severe and may cause complete obstruction of the cerebral aqueduct either by plugging with necrotic debris or by intense reactive glial proliferation. The organisms may be in the encysted form, which causes little tissue reaction, or scattered individually when they incite granuloma formation (Fig. 27.25). Microgyria and subependymal cysts may also be seen (Friede 1989d).

Bacterial Infections

Bacterial infections of the brain cause meningitis and ventriculitis. Transplacental infection may occur but purulent leptomeningitis in utero is very rare.

Bacterial meningitis is more common in the first months of life than at any other time, and three quarters of cases of meningitis are in children under 5 years of age. Meningitis in the first week is due to prenatal infection, almost always with group B streptococcus or *Escherichia coli,* while meningitis presenting after the first week is postnatally acquired and a variety of other organisms (e.g., staphylococci, *Klebsiella,* and *Pseudomonas*) may be responsible.

The reasons for this pattern of infections are unknown but may include immaturity of the neonatal immune system with inefficient neutrophil recruitment or age-dependent specific vulnerability to infection. Studies on immature rat brain have demonstrated a "window of vulnerability" of a few weeks shortly after birth when intense inflammation and increased permeability of the blood–brain barrier can be induced by cytokine injection, effects not seen before or after this period (Anthony et al. 1996). If such a window exists in the human infant, it would explain the increased susceptibility to infection and the severity of the resulting disease at certain developmental stages. Passive immunity from selective transplacental transfer of antibodies may confer immunity to specific organisms in the first weeks of life.

The macroscopic features of purulent bacterial meningitis in the neonate are well known. In early cases the meninges may be infected but not purulent and the diagnosis may not be apparent. In later stages the purulent exudate is obvious (Fig. 27.26A). In the immature brain with shallow cerebral sulci the leptomeninges frequently tear off during brain removal and processing. A block for diagnosis should include the depths of a sulcus or the cerebellum, in which remnants of the meninges tucked deep into sulci survive processing.

Bacteria invade the CNS from the bloodstream. They first localize in the choroid plexus, then the ventricular system, and pass into the subarachnoid space. Ventriculitis is common in neonatal

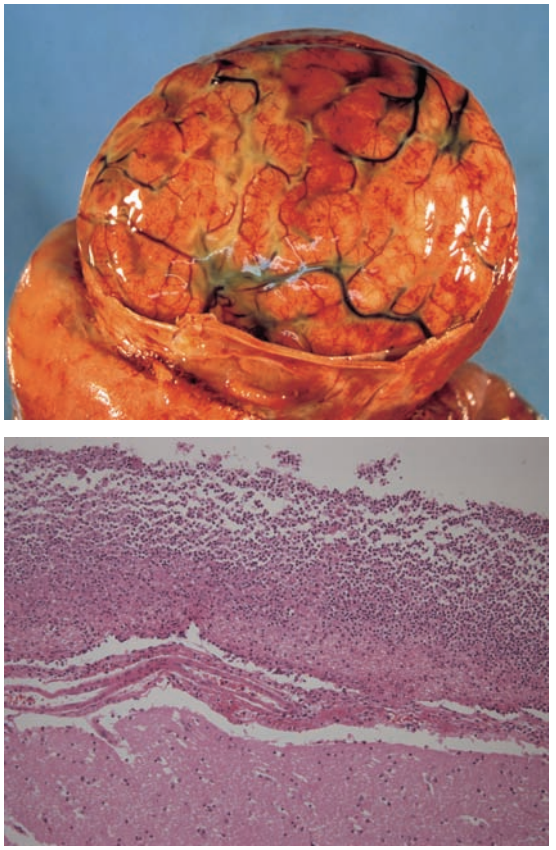


Figure 27.26. (A) Acute bacterial meningitis, term infant died at 3 weeks, group B streptococcus (GBS) isolated. There is brain swelling, leptomeningeal congestion, venous dilatation, and purulent exudates. (B) High-power micrograph showing a prominent polymorph infiltrate of the leptomeninges (H&E).

infections. The brain becomes edematous and congested with narrowing of the ventricular system.

In the first week polymorphs are prominent in the leptomeninges but later mononuclear cells predominate (Fig. 27.26B). Perivascular cuffs of inflammatory cells surround superficial vessels dipping into the cortex. Obstruction of superficial veins by thrombosis in the early stages of the disease causes hemorrhagic infarcts in 30% of cases (Friede 1989e). Deep, white matter infarcts due to thrombosis of subependymal veins are also seen. Meningeal inflammation may fill the sleeves surrounding cranial nerve roots, causing compression and cranial neuropathy.

The long-term complications of meningitis include hydrocephalus and multilocular cystic cavitation of the brain. Hydrocephalus follows fibrosis of the inflamed meninges, which prevent either drainage of CSF from the fourth ventricle or its resorption by arachnoid villi. Hydrocephalus limited to the lateral and third ventricles results from obstruction of the cerebral aqueduct.

Listeria Meningitis

Listeria septicemia acquired in utero usually involves the CNS, causing meningoencephalitis. Granulomas form around blood vessels in the leptomeninges, ependyma, and choroid plexus (Fig. 27.27). The granulomas may be small and infrequent, and examination of a large number of blocks is necessary to establish the diagnosis. In some cases small disseminated foci of necrosis are also found in the brain parenchyma.

Fungal Infections

Candidiasis

This is the most common infection of the brain in the neonatal period. The incidence is particularly high in sick neonates, requiring prolonged hospi-

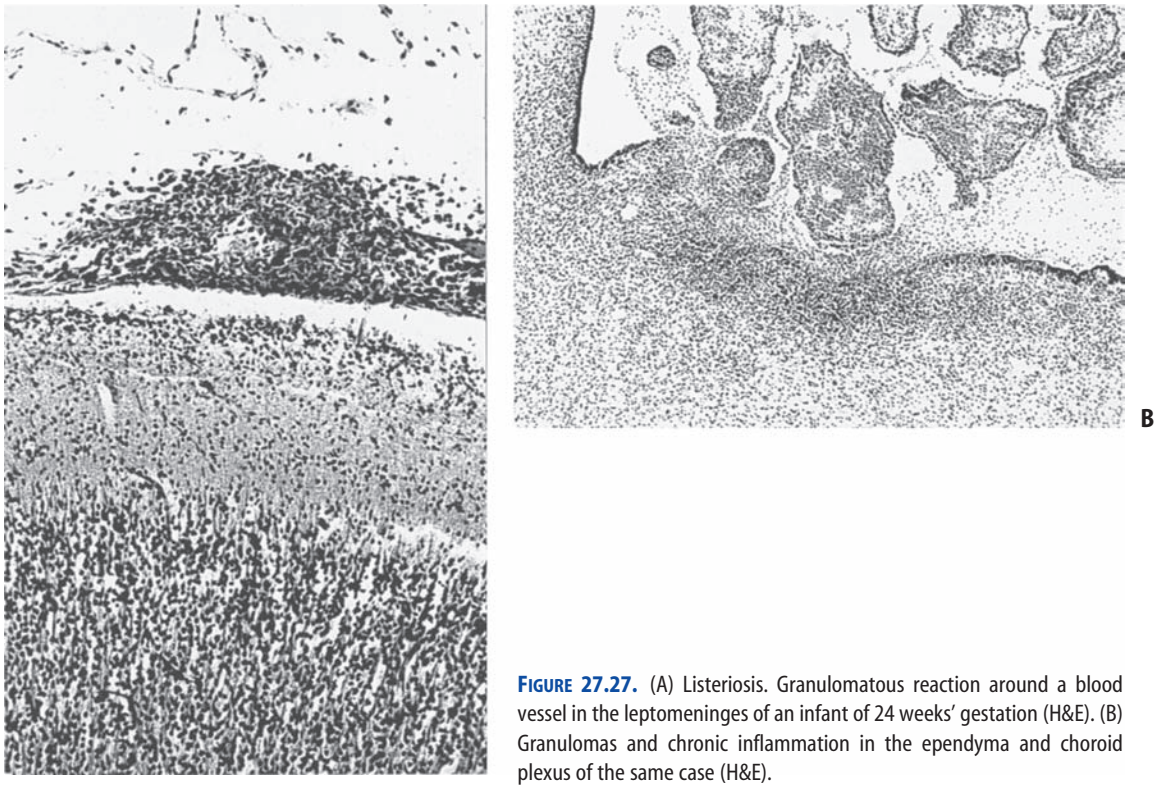


Figure 27.27. (A) Listeriosis. Granulomatous reaction around a blood vessel in the leptomeninges of an infant of 24 weeks' gestation (H&E). (B) Granulomas and chronic inflammation in the ependyma and choroid plexus of the same case (H&E).

talization, who are treated with broad-spectrum antibiotics that deplete the normal flora.

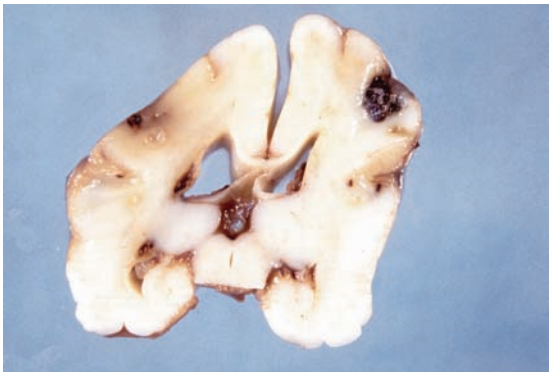
Candidiasis can cause meningitis and ventriculitis or more widespread infection of the cerebral parenchyma. Multiple yellow lesions, sometimes cystic, are present throughout all areas of the cerebral parenchyma (Fig. 27.28A). Histology shows necrosis with a granulomatous response and reactive gliosis (Fig. 27.28B). Organisms are demonstrated in these lesions with periodic acid-Schiff (PAS) or Grocott stain and show small colonies of short, budding hyphae or yeast forms (Fig. 27.28C).

Metabolic Disorders

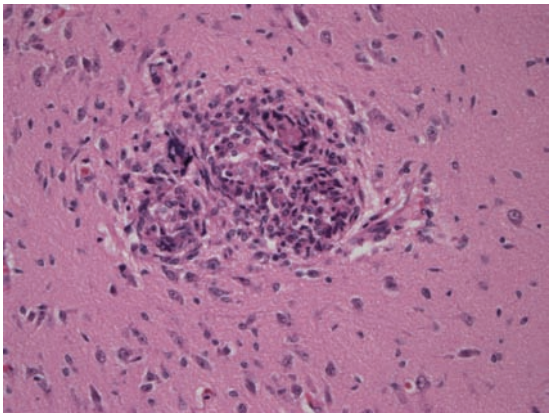
The neuropathology of metabolic disease is not strictly within the scope of this chapter, but a number of metabolic disorders, particularly those of amino acid and organic acid metabolism, cause similar changes in the brain, namely astrocytosis, spongy change of the white matter, and reduction in amount of myelin (Martin and Schlote 1972; Agamanolis 1982). In others malformations may be seen (Kolodny 1989). The reader is referred to a review of neurometabolic disorders by Moser (1998).

Kernicterus

This condition, now rarely seen due to improved antenatal and neonatal measures, has a characteristic macroscopic appearance. There is selective yellow pigmentation of basal ganglia, particularly globus pallidus, dentate nucleus in the cerebellum, and brainstem nuclei by bilirubin deposition (Fig. 27.29). Histologically there is swelling of affected neurons, sometimes with obvious cytoplasmic yellow pigment, although this is often lost with formalin fixation. The cytoplasm is vacuolated. The neurons subsequently degenerate, resulting in reactive gliosis. For a detailed account of the biochemistry, neuropathology, and clinical features the reader is referred to Friede (1989b) and Volpe (2001h).



A



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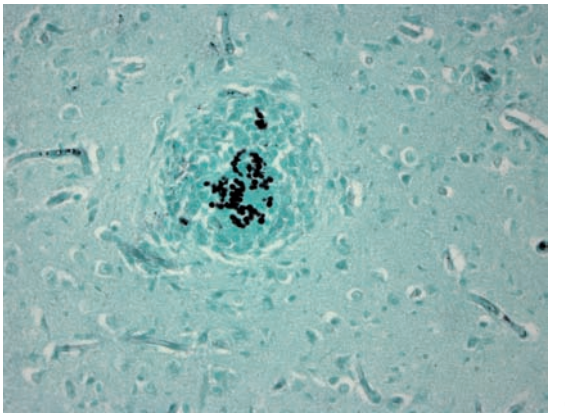

C

Figure 27.28. (A) Coronal section of a brain with candida septicemia and multiple candida abscesses. The abscesses are hemorrhagic and necrotic. (B) High-power micrograph showing a granuloma including multinucleated giant cells. Pale inclusions could be seen in the cytoplasm of the giant cells (H&E). (C) A Grocott stain highlights the candida yeast forms. They are particularly prominent in the cytoplasm of giant cells.

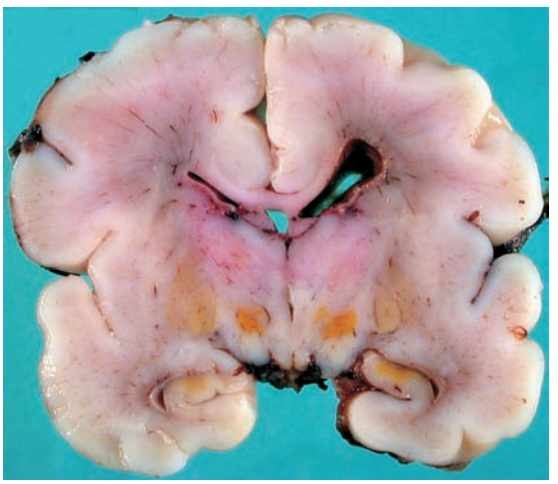

Figure 27.29. Kernicterus. There is yellow discoloration of deep gray nuclei and the hippocampus within the cerebral hemispheres.

Mitochondrial Diseases

Mitochondrial diseases, due to mutations and deletions in mitochondrial DNA, have been widely studied in the last decade. These defects lead to disruption of energy metabolism with a wide spectrum of clinical presentations. Leigh's disease (subacute necrotizing encephalomyelopathy) presents at birth or in the neonatal period and is due to a group of diverse genetic and biochemical defects mainly involved in mitochondrial energy generation (Brown and Squier 1996). The characteristic neuropathological features are of symmetrical necrotic lesions typically involving the basal ganglia, brainstem, cerebellum, and spinal cord (Fig. 27.30). Microscopically there is capillary proliferation and patchy tissue loss with diffuse astrocyte proliferation throughout the white matter. Two siblings have been reported with extensive cystic damage and calcification of fetal origin, resembling intrauterine infection, but

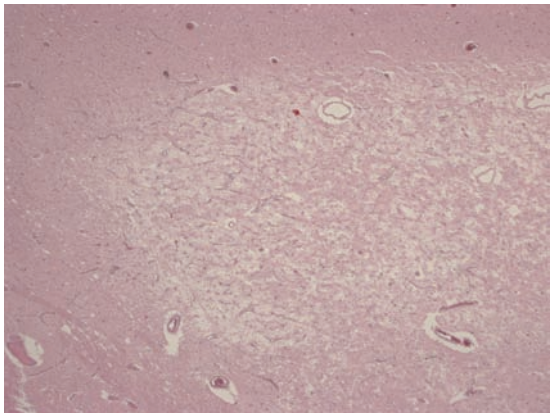

Figure 27.30. Low-power micrograph of the cerebellum from a case of Leigh's disease. There is necrosis of the dentate nucleus with reactive gliosis and capillary proliferation.

in whom defects of the mitochondrial respiratory chain were demonstrated (Samson et al. 1994). Pyruvate dehydrogenase deficiency is associated with disorders of neuronal migration, agenesis of the corpus callosum, and cystic lesions in the white matter (Brown and Squier 1996).

Other Metabolic Conditions

Other metabolic conditions may rarely present as a fatal condition in the neonatal period. Peroxisomal disorders, such as Zellweger's syndrome, often have prominent neuronal migration disorders, myelin loss, and neuronal loss (Zellweger 1987). Disorders of amino acid metabolism can present in the neonatal period. Usually the brain is swollen, although intrauterine cystic necrosis of the brain has been described in hereditary ornithine transcarbamylase deficiency (Filloux et al. 1986).

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28
Skeletal Muscle and Peripheral Nerves

Anthony J. Bourne and Nicholas D. Manton

Development of Skeletal Muscle

The skeletal muscles derive from embryonic mesenchyme. The trunk musculature mainly derives from paraxial mesoderm, which segments into somites. The dorsal part of the somite, the dermomyotome, gives rise to the skeletal muscles of the body and limbs. The muscles of the head and neck may derive from anterior, segmented, paraxial mesoderm or from the branchial arches. A number of regulatory factors involved in this process have now been identified, and the molecular mechanisms involved are now becoming better understood. A recent review outlines the role played by several factors including Pax3, c-met, Lbx1, Mox2, Myf5, MyoD, Myogenin, Mrf4, and Mef2 (Buckingham et al. 2003).

The formation of a skeletal muscle from undifferentiated cells occurs in three phases: hyperplasia, metabolic differentiation, and volumetric growth of the muscle fibers. The initial phase of hyperplasia is characterized by the differentiation of undifferentiated muscle precursors into myoblasts, followed by several cycles of cell division with the fusion of myoblasts into myotubes. These are elongate structures containing several centrally located nuclei with peripheral myofilaments. As further myofilaments are laid down, the nuclei become displaced to the periphery of the muscle fiber, leading to the development of the mature myofiber with several peripheral nuclei and a sarcoplasm with evenly dispersed myofibrils. This development of myofibers is generally completed at between 18 and 20 weeks' gestation.

Skeletal muscle is a specialized tissue composed of several different types of muscle fibers. These fibers differ in their physical properties (speed of contraction, resistance to fatigue) and in the composition of their contractile proteins. Enzyme histochemical studies of oxidative enzymes and myofibrillar adenosine triphosphatase (ATPase) and immunocytochemical studies using antibodies specific for different myosin isoforms have made it possible to categorize at the light microscope level the slow (type I) and fast (type II, with subgroups IIA, IIB, and IIC) contracting muscle fibers. During early fetal development all fibers are undifferentiated, having the characteristics of type IIC fetal fibers, which are progressively replaced by mature type I and II fibers. Histochemical differentiation begins with the appearance of the first type I fibers at between 16 and 20 weeks' gestation and ends when the last type IIC fibers disappear at or soon after birth. At birth, there are approximately 40% type I and 60% type II fibers, with the proportions varying from muscle to muscle. The reactivities of the oxidative enzyme nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) also evolve during fetal development due to the progressive organization of the myofibrils and mitochondria. In early development, the fetal fibers show central reactivity with a peripheral "halo" of reduced reactivity, but, by the time of birth, maturation of the fibers leads to relatively uniform staining. At birth, the majority of muscles have attained their mature histochemical and immunocytochemical phenotype, which is completed in the first few months after birth, resulting in the characteristic

checkerboard pattern of fiber staining in myofibrillar ATPase preparations. The quadriceps muscle, which is generally used as a reference muscle in pathology, is made up of fibers with a regular diameter of 12 to 15 μm, with the diameter increasing by approximately 2 μm per year until the age of 14 or 15 years, when the adult norm of approximately 40 μm is reached.

During muscle development, membrane-related proteins such as dystrophin and merosin start to be synthesized in early myotubes and can be detected immunohistochemically from approximately 15 weeks' gestation in the fetal muscle once they have become organized in the region of the cell membrane.

Neuromuscular Disorders Affecting the Fetus and Neonate

The majority of neuromuscular disorders presenting in the fetal or neonatal period are genetically determined, and there have been major advances in recent years in the ability to identify the underlying genetic abnormalities. In many cases diagnosis is now based on genetic studies rather than interpretation of morphological changes on muscle or peripheral nerve biopsy. The expanding field of genetic investigative techniques has brought with it the possibility of prenatal diagnosis. In addition, advances in antenatal radiology [ultrasound and magnetic resonance imaging (MRI)] have also resulted in earlier identification of features suggesting a congenital neuromuscular disorder, enabling prenatal genetic investigations, and giving parents the option of terminating the pregnancy. The role of the pathologist has therefore changed somewhat with a greater emphasis on fetal autopsy diagnosis. However, there are still neonatal cases (predominantly involving hypotonic infants) requiring expert interpretation of muscle biopsies in the diagnostic workup.

The importance of a systematic approach in the diagnosis of a congenital neuromuscular disorder cannot be overemphasized. Arriving at the correct diagnosis is often a lengthy process, requiring the use of a wide variety of diagnostic modalities. Many of these investigative techniques (such as metabolic screening and molecular studies) are expensive and labor intensive with results taking up to many months to achieve. It is therefore vital that the diagnostic workup uses a systematic approach in order first to reach the correct diagnosis and second to avoid requesting inappropriate investigations. In cases that come to autopsy, it is self-evident that there is only one opportunity to take appropriate samples for histological, metabolic, and genetic investigations, so the planned approach must be carefully considered, and consultation should be sought from a clinical geneticist and metabolic pediatrician if there is any doubt regarding the specific tests that may be appropriate. It also needs to be recognized that even after an exhaustive clinicopathological workup, an underlying cause may not be found in some cases.

Congenital neuromuscular disorders are a heterogeneous group of processes involving disruption of any of the components of the neuromuscular pathway including the brain, anterior horn cells of the spinal cord, nerve roots, peripheral nerves, motor end plates, and the muscles themselves. These disorders, therefore, may be grouped into the following categories:

1. Congenital neurological disorders (either central in origin or due to inherited peripheral nerve diseases)
2. Congenital myopathies and muscular dystrophies (many of which now have known causative genetic abnormalities for which specific tests are commercially available).
3. Congenital myasthenic syndromes (either related to maternally derived factors interfering with fetal neurotransmitters or due to inherited genetic disorders of the neuromuscular junction).
4. Congenital connective tissue disorders (including congenital restrictive dermopathy).
5. Limitation of space in utero resulting in restriction of fetal movement (such as in the oligohydramnios sequence)
6. Maternally derived inflammatory disorders (due to viral infection or autoimmune disease)

The common denominator among these disorders is limitation of fetal movement in utero. Affected fetuses or infants generally present either with multiple congenital contractures (arthrogry-

posis) or as congenital hypotonia (the "floppy infant"). The phenotype in part appears to depend on the in-utero timing of onset of the underlying disorder. This timing seems to be an important determinant of the severity and distribution of joint contractures and the secondary changes affecting other organ systems (such as pulmonary hypoplasia) (Hall 1986). It seems that lack of fetal movement takes over a month to produce contractures in the third trimester but the length of time required in first and second trimester may be less (Hall 2002).

The Concept of Fetal Akinesia and the Pena-Shokeir Phenotype

The Pena-Shokeir syndrome was first described in 1974 in two siblings who died with multiple contractures, facial abnormalities, camptodactyly, and pulmonary hypoplasia (Pena and Shokeir 1974). Pena and Shokeir concluded that this was a lethal syndrome with autosomal recessive inheritance. Subsequently, several subtypes of the syndrome were reported by other authors who described similar clinical features but different pathological findings (Chen et al. 1983; Herva et al. 1985; Lindhout et al. 1985). Hall (1986) suggested that this was not a syndrome but represented a phenotype with heterogeneous causes. Moessinger (1983) suggested the term *fetal akinesia deformation sequence* based on animal studies following curarization of rats, and this term has gained wider acceptance and use in recent years. It must be stressed, however, that the terms *fetal akinesia* or *fetal akinesia deformation sequence* (FADS) do not represent a specific diagnosis but rather describe a phenotype with heterogeneous causes. Fetal akinesia deformation sequence is different from arthrogryposis in that there are additional features to multiple joint contractures (including polyhydramnios and pulmonary hypoplasia) in FADS.

The potential causes of the fetal akinesia deformation sequence are varied and include virtually all of the diagnostic groups described elsewhere in this chapter, such as neurogenic or myopathic disorders, intrauterine constraint, and restrictive dermopathy. A definite cause despite extensive investigation may not always be determinable. In a study by Witters et al. (2002) of 30 consecutive in utero diagnoses of FADS utilizing prenatal ultrasound imaging and fetopathological examination, a definite cause was found in no more than half of cases.

Arthrogryposis

Arthrogryposis multiplex congenita (AMC) is a term that has been used historically to describe conditions manifesting as nonprogressive multiple congenital joint contractures. There has been much confusion in the literature regarding this condition as it has been poorly defined, and many authors do not adequately describe what is meant by the term. Arthrogryposis multiplex congenita is rare, with an approximate incidence of 1 in 3000, while congenital contractures such as talipes equinovarus are relatively common, with 1 in 100 to 1 in 200 babies born with some form of contracture (Hall 2002). The entity of AMC suggests a generalized condition involving more than one part of the body. It is no longer regarded as a diagnosis in itself but rather as a descriptive term with multiple potential causes, all of which result in multiple congenital joint contractures, typically occurring in early pregnancy. For normal development, the fetus needs to be able to move freely from 7 to 8 weeks of gestation onward (Porter 1995), and anything that impairs movement from that time may lead to development of contractures. The list of disorders resulting in the arthrogryposis phenotype is therefore long and diverse, including such entities as amyoplasia ("classical arthrogryposis"), the multiple pterygium syndromes, the distal arthrogryposes (of which there are multiple types), congenital inflammatory myopathies, conditions resulting in intrauterine constraint (most commonly oligohydramnios), and the rare connective tissue disorders such as restrictive dermopathy. It has been estimated that 90% to 95% of cases of arthrogryposis are broadly neurogenic in origin, while 5% to 10% of cases have been considered to be myopathic (Gordon 1998).

The congenital muscular dystrophies and congenital myopathies most commonly present with weakness and hypotonia and, although contractures may also be a feature (particularly in

the case of congenital myotonic dystrophy), an arthrogrypotic phenotype is rare. These disorders are discussed separately.

Amyoplasia

Amyoplasia is the most common condition resulting in severe multiple congenital joint contractures and is responsible for what the majority of clinicians understand by the term *arthrogryposis*. The incidence is reported as 1 in 10,000 live births, and all cases have been sporadic (Hall 2002). It is said to be responsible for approximately one third of all cases of arthrogryposis (Hall 2002). Amyoplasia characterizes patients with a congenital absence of limb musculature and replacement by fatty and fibrous tissue (Hall et al. 1983a,b). The contractures are bilateral and symmetrical. There is almost always talipes equinovarus and flexion deformities of the wrists. Most commonly, the shoulders are internally rotated and the elbows are in fixed extension. The hips may be extended or flexed in abduction or adduction, and the knees may be flexed or extended. Pterygia are not usually prominent. Additional features include micrognathia and the frequent occurrence of midline frontal capillary hemangiomas (Banker 1986; Hall 2002). In the vast majority of cases, there is no involvement of other organ systems, with only a minority of cases having abdominal wall defects or intestinal atresia (Hall 2002). Intelligence is usually normal.

The pathology is characterized by replacement of limb muscles with fatty and fibrous tissue. The interpretation of these appearances depends to some degree on the gestation or age of the patient and on the muscle groups examined, as the pattern and severity may vary between muscle groups. In the case of the normal fetus, muscle fibers are small and rounded as they are in arthrogryposis, and this may cause some confusion in interpretation. This is not such a problem in the infant, as small rounded muscle fibers are never normal. It is therefore important in the case of the fetus to examine sufficient muscle tissue to appreciate the additional abnormalities seen in amyoplasia. Muscle fasciculi are incomplete with portions of, or whole, fasciculi replaced by adipose tissue (Figs. 28.1 and 28.2). The fasciculi are surrounded by perimysium, said to be a characteristic feature

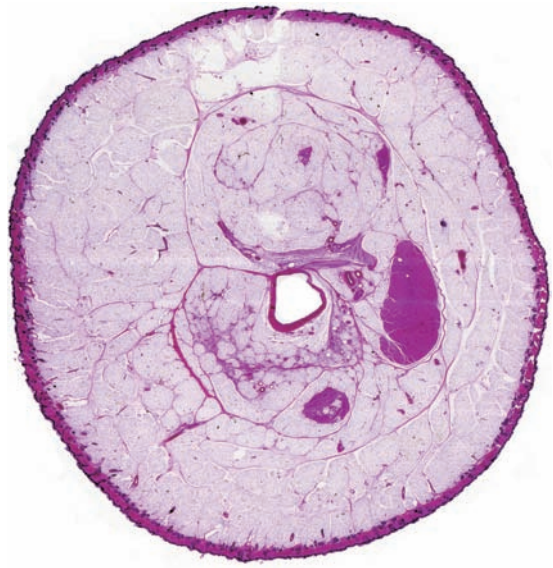

Figure 28.1. Whole-mount section from the thigh of a neonate with amyoplasia showing almost complete replacement of muscle compartments by adipose tissue.

(Banker 1986). The abnormalities are generally most marked in muscles opposing the contracture; for example, in flexion contracture of the elbow, the changes are best demonstrated in the triceps muscle. In autopsy cases, it is apparent that muscle samples should include prime mover muscles as well as opposing muscle groups.

The pathogenesis of amyoplasia is not entirely clear. Banker (1986) has suggested that amyopla-

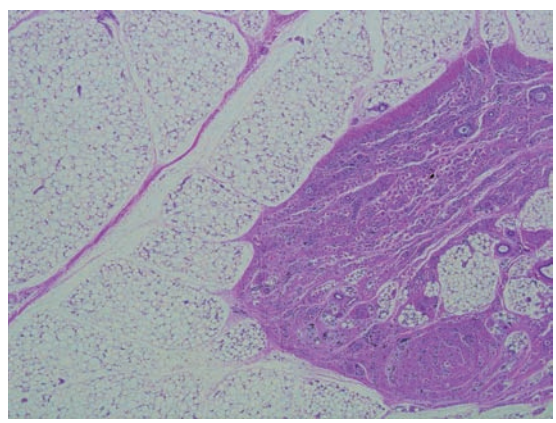

Figure 28.2. Mature adipose tissue with adjacent isolated muscle fascicle in amyoplasia (same case as depicted in Figure 28.1; original magnification ×100).

sia is due to a primary defect in the anterior horn cells of the spinal cord early in development (so-called anterior horn cell dysgenesis). The findings are characterized by depletion and disorganization of the motor neurons, particularly in the cervical and lumbosacral regions of the cord. In contrast to infantile spinal muscular atrophy (Werdnig-Hoffmann disease), there is no associated gliosis in affected areas of the cord. The extent of the lack of muscle and the replacement by fat are said to be good indicators of the degree of anterior horn neuronal cell dysgenesis of the spinal cord (Banker 1986). With regard to interpretation of the spinal cord abnormalities, there is little morphometric data in the literature documenting normal values for anterior horn neuron counts (Clarren and Hall 1983), which can make interpretation difficult.

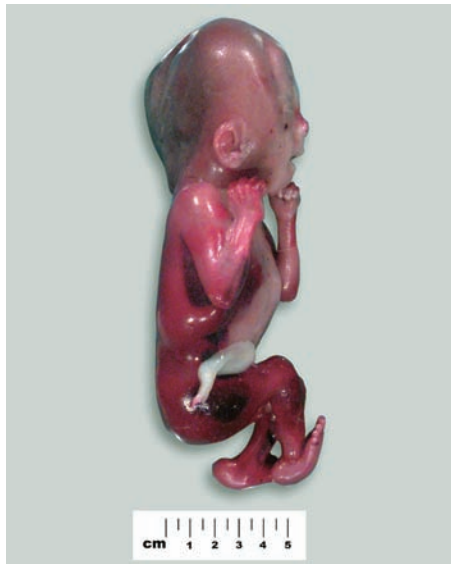

Figure 28.3. Fetus with arthrogryposis due to spinal cord anomaly. Note the contractures and prominent rocker-bottom feet.

Other Central Nervous System Disorders

Apart from spinal cord neuronal dysgenesis, a variety of other central nervous system disorders have been associated with arthrogryposis. These include polymicrogyria where there is an abnormality of neuronal migration at the level of the cerebral cortex, which may be associated with similar neuronal migration disorders involving the brainstem and spinal cord (Banker 1986). Neuronal migration disorders may form part of a wider systemic disorder such as Zellweger syndrome (cerebrohepatorenal syndrome). Other central nervous system abnormalities found in Zellweger syndrome include hydrocephalus, thinning of the corpus callosum, and cerebellar hypoplasia (Banker 1986). Arthrogryposis, predominantly involving the lower limbs, has also been described in lumbosacral myelomeningocele, and contractures have been observed in anencephaly associated with dysgenesis of the anterior horns (Banker 1985). Arthrogryposis may also be seen in structural abnormalities of the spinal cord (Figs. 28.3 and 28.4).

The Multiple Pterygium Syndromes

Many babies with arthrogryposis have pterygia (or webs) across the contracted joints. However, there are a group of syndromes in which a dominant feature is marked pterygia formation. Several of these syndromes, such as multiple pterygium syndrome (Escobar type), popliteal pterygium syndrome (Gorlin type), and multiple pterygium syndrome with malignant hypertension, are nonlethal with generally autosomal recessive inheritance patterns, while Bartsocas-Papas syndrome (lethal popliteal web syndrome) is autosomal dominant (Hall 2002). These conditions are rarely encountered by the perinatal pathologist and are

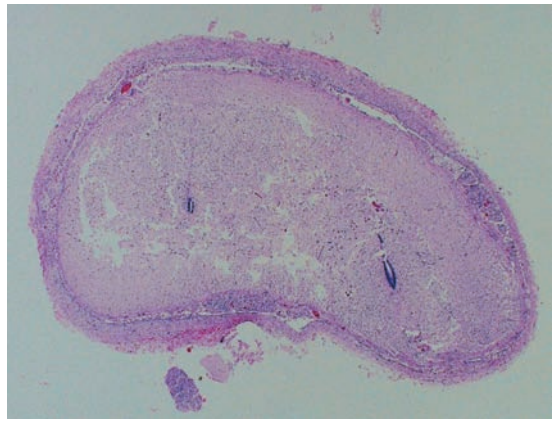

Figure 28.4. Spinal cord anomaly in a case of arthrogryposis. Note the presence of two ependymal-lined spinal canals (same case as shown in Figure 28.3).

not discussed further here. The disorder most likely to be encountered in perinatal pathology is the lethal multiple pterygium syndrome.

Lethal multiple pterygium syndrome (LMPS) encompasses multiple congenital joint contractures with joint pterygia and in utero lethality in the second or third trimester of pregnancy (Cox et al. 2002). Affected fetuses are also typically growth restricted and hydropic (or with a cystic hygroma), and have hypoplastic lungs and cleft palate (Cox et al. 2002; Hall 2002). Other abnormalities include cardiac hypoplasia, ambiguous genitalia, hypertelorism, and polyhydramnios (Froster et al. 1997). The pathogenesis of LMPS has been suggested as a combination of lack of fetal movement in early gestation with the effects of early-onset hydrops (Moerman et al. 1990). Hall (1984) suggested that LMPS could be subdivided into three different types based on the presence or absence of cartilaginous joint fusions in the spine or limbs, but this has not been universally accepted due to the difficulty in interpreting such radiological changes at different gestational ages. Inheritance is generally reported as autosomal recessive (Hall 2002), but an X-linked recessive form has been suggested (Tolmie et al. 1987). Prenatal ultrasound diagnosis is possible, as the abnormalities are detectable in the second trimester (Martin et al. 1986).

The neuromuscular pathology of LMPS has been poorly described in the literature. However, Cox et al. (2002) showed marked heterogeneity of muscle pathological abnormalities. In all cases, there was reduced muscle bulk, but on histology the appearances varied and included features of vacuolar degeneration, dystrophy (with variation in muscle fiber diameter and interstitial fibrosis), myotubular morphology, and generalized hypotrophy, while in one case the appearances were essentially normal. Cox et al. also identified cases with central nervous system anomalies including agenesis of the corpus callosum and patchy dysplasia of the cerebral cortex. However, examination of the spinal cord did not show depletion or degeneration of the anterior horn cells. It was therefore suggested that LMPS is not a distinct syndrome but a phenotypic expression of severe early-onset fetal neuromuscular disease manifesting as fetal akinesia in the first or early second trimester.

It is clear that our understanding of LMPS is still evolving, but at present it seems that parents can be counseled along the lines of autosomal recessive inheritance and offered antenatal ultrasound diagnosis in future pregnancies.

The Distal Arthrogryposes

The distal arthrogryposes are a heritable group of disorders characterized by congenital nonprogressive contractures of two or more body areas without primary neurological or muscle disease and featuring a consistent pattern of predominantly distal joint involvement. They have an autosomal dominant inheritance pattern with widely variable clinical expressivity. An initial classification by Hall et al. (1982) was revised by Bamshad et al. (1996) with recognition of 10 such syndromes classified according to the proportion of features shared in common. The prototypic distal arthrogryposis (DA) is type 1 [DA1, On-Line Mendelian Inheritance in Man (OMIM) number 108120]. This type is primarily characterized by camptodactyly and clubbed foot. There are no associated visceral abnormalities; intelligence is normal, and response to physical therapy is good. Other forms of DA show similar patterns of contracture but with additional abnormalities. For example, in Freeman-Sheldon syndrome (DA2A, OMIM 193700) additional features include scoliosis, a small oral orifice, H-shaped chin dimpling, deep nasolabial folds, and narrow palpebral fissures. A variant syndrome of Freeman-Sheldon syndrome (DA2B, OMIM 601680) has also been described. Other subtypes include forms with cleft palate (Gordon syndrome, DA3, OMIM 114300), severe scoliosis (DA4, OMIM 609128), and oculomotor limitation (DA5, OMIM 108145). The distal arthrogryposes may be caused by mutations in genes encoding fast-twitch contractile proteins (Sung et al. 2003).

Conditions Resulting in Intrauterine Constraint

During development, morphogenesis takes place in a restricted environment with the amniotic fluid protecting the fetus from uterine wall pressure and allowing fetal movement to occur. The association of limb contractures, large hands, flat-

tened nose, epicanthic folds, abnormally formed ears, and pulmonary hypoplasia with oligohydramnios was first described by Potter in 1965. In the original case series, the underlying cause for the absent amniotic fluid was renal agenesis. However, any condition leading to prolonged exposure to reduced amniotic fluid volume will cause similar findings (also known as the oligohydramnios sequence). The reduced amniotic fluid may be due to premature rupture of membranes or to abnormalities of the fetal urinary tract. Features distinguishing oligohydramnios sequence (OS) from fetal akinesia due to other neuromuscular disorders include the frequent asymmetrical nature of the postural abnormalities in OS (Fig. 28.5). Because immobilization in OS occurs later in gestation after muscular development has already occurred, bone mass is usually normal, whereas in other causes of fetal akinesia, bone mass is reduced (Hammond and Donnenfeld 1995). Micrognathia is not usually as marked in OS as swallowing movements are preserved. Mandibular abnormalities tend to manifest more as asymmetry due to fetal constraint (Rodriguez and Palacios 1991).

In addition to OS, other causes of intrauterine fetal compression include maternal leiomyomas and müllerian abnormalities such as bicornuate uterus (Hammond and Donnenfeld 1995). It is apparent that when investigating the fetus with contractures, a full pregnancy history (specifically including a history of premature rupture of membranes or uterine malformations) and autopsy examination of the urinary tract are most important.

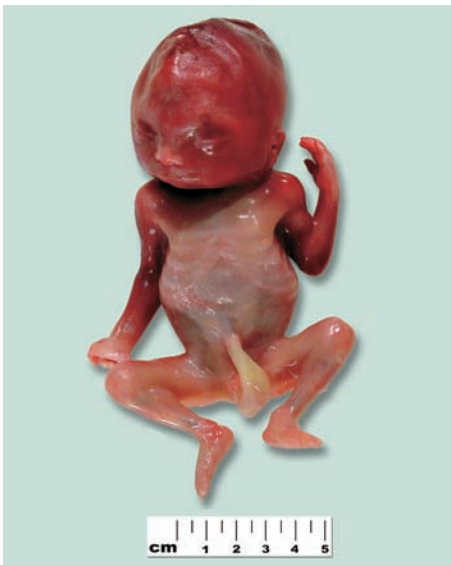

Figure 28.5. Case of arthrogryposis due to prolonged reduction of amniotic fluid volume. The contractures are asymmetrical, particularly those involving the upper limbs.

Restrictive Dermopathy

Restrictive dermopathy (Witt et al. 1986) (OMIM 275210) is a rare and lethal autosomal recessive genodermatosis. It results in abnormal differentiation of skin, bone, and lungs leading to rigidity and tautness of the skin. Features of fetal akinesia result, with multiple joint contractures, polyhydramnios, pulmonary hypoplasia, and bone abnormalities including widened fontanelles and dysplasia of the clavicles. The mouth is fixed open in an "O" position. Histological abnormalities of the skin include a thinned hyperkeratotic epidermis with paucity and hypoplasia of skin appendages. There is also deficiency of dermal elastin and prominence of abnormally dense dermal collagen bundles (Mau et al. 1997; Wesche et al. 2001; Nijsten et al. 2002; Navarro et al. 2004). These abnormalities usually appear after 22 to 24 weeks' gestation, which is why prenatal ultrasound detection may fail, given that routine scanning is usually performed prior to this. Navarro et al. (2004) recently identified mutations in the *LMNA* and *ZMPSTE24* genes located at 1p34. Both of these genes are involved in the production of nuclear lamins, suggesting a final common pathogenetic pathway with defects of the nuclear lamina and matrix involved in restrictive dermopathy.

Congenital Inflammatory Myopathies

Congenital inflammatory myopathy refers to cases in which there is muscle biopsy evidence of inflammation and a prenatal presentation (Shevell et al. 1990) (Figs. 28.6 and 28.7). It has been recognized that muscle inflammation is a nonspecific finding in several of the congenital muscular dystrophies such as Fukuyama muscular dystrophy, Walker-Warburg syndrome, and congenital muscular dystrophy with laminin α2 (merosin) deficiency (McNeil et al. 2002). The entity has been

FIGURE 28.6. Fetus with arthrogryposis due to inflammatory myopathy. In this case, the contractures are symmetrical.

Primary Fetal Neuromuscular Disorders

Primary neuromuscular disorders, with some notable exceptions, uncommonly present in the fetal or neonatal period. Although a later presentation with delayed development of motor skills is more common, neuromuscular disorders may present in the fetal or the neonatal period generally as a result of multiple joint deformities (arthrogryposis multiplex congenita) or congenital hypotonia. While most hypotonic infants do not have a primary muscular disorder, but suffer from some central nervous system (or systemic) disorder, such as hypotonic cerebral palsy, these cases need to be distinguished from those with a neuromuscular disorder, and in the latter group the precise cause needs to be defined. As most of these defined disorders have a genetic basis, accurate diagnosis is important not only to establish the cause of disease in the individual but also as the basis for providing the family with precise genetic counseling. Some neuromuscular disorders may present with a variable clinical picture with features of both joint deformity and hypotonia, and where one or the other of these presenting symptoms may predominate in the individual patient.

the subject of controversy, given the lack of diagnostic criteria, the presence of inflammatory changes in biopsies from children with other specific disorders, and the uncertainty regarding pathogenesis. Possible causes include in utero bacterial or viral infection (McNeil et al. 2002) and maternal autoimmune disease.

In an attempt to refine diagnostic criteria for congenital inflammatory myopathy, McNeil et al. (2002) proposed a diagnostic schema with major criteria including the presence of inflammatory infiltrates in muscle, absence of dystrophic and congenital myopathic changes, and normal immunohistochemical staining for merosin. Minor criteria included elevated serum creatine kinase (CK) activity, electromyograph (EMG) findings including fibrillations/positive sharp waves, and hypotonia or weakness.

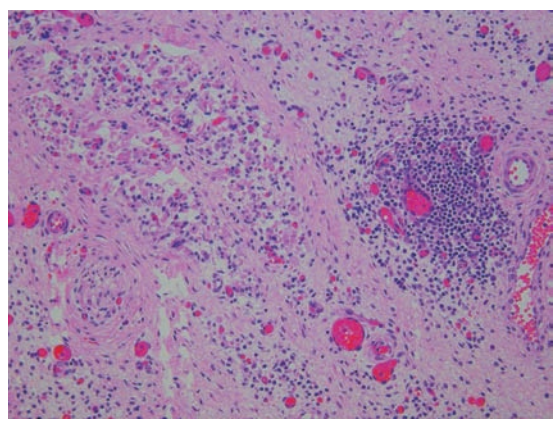

FIGURE 28.7. Inflammatory myopathy. There is atrophy of muscle fibers with increased interstitial connective tissue and a lymphocytic inflammatory cell infiltrate (same case as shown in Figure 28.6; original magnification ×200).

Severe neuromuscular disorders presenting during intrauterine life often result in oligohydramnios and a decrease in fetal movements. In the neonatal period hypotonia and muscle weakness may be evidenced by respiratory distress or swallowing difficulty.

The disorders to be considered in the differential diagnosis generally fit into four groups: congenital myopathy, congenital muscular dystrophy, infantile spinal muscular atrophy, and congenital myotonic dystrophy. Other disease categories to be discussed include the neuromuscular transmission disorders, the metabolic myopathies, and the peripheral neuropathies. The dystrophinopathies (including Duchenne muscular dystrophy and Becker muscular dystrophy) are not symptomatic in the fetal or neonatal period and are not described here. The reader is referred to detailed muscle pathology texts for information on these disorders (Dubowitz 1995).

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is generally characterized by progressive muscle weakness because of loss of anterior horn cells in the spinal cord and brainstem nuclei. Typically, the condition has been classified into three groups:

SMA I: onset before 6 months of age (infantile SMA, Werdnig-Hoffmann disease), with the patients presenting with severe muscle weakness with facial sparing
SMA II: onset between 6 and 18 months (intermediate SMA, Dubowitz disease)
SMA III: onset in childhood and adolescence (juvenile SMA, Kugelberg-Welander disease)

It has become clear that the spectrum includes patients with prenatal onset with severe joint contractures at birth (SMA 0) and even with presentation in adult life (SMA IV).

Spinal muscular atrophy is inherited in an autosomal recessive fashion, and it is known that the phenotype of SMA is associated with disease-causing mutations of the survival motor neuron (*SMN1*) gene located at 5q12.2-q13.3. The SMN region on chromosome 5 is unusually complex, and the *SMN2* gene, a nearly identical copy of the *SMN1* gene, is located in tandem with the *SMN1* gene. The complexity of the region makes molecular analysis difficult to perform and interpret. About 95% of individuals with SMA are homozygous for absence of exons 7 and 8 of *SMN1*, and about 5% are compound heterozygotes for absence of exons 7 and 8 of *SMN1* and a point mutation in *SMN1* (Mailman et al. 2002). Confirmation of the diagnosis is now available by molecular testing, and carrier detection and antenatal diagnosis are also available in specialist centers.

Diagnosis by examination of skeletal muscle is straightforward, with the typical appearance of large group atrophy with scattered enlarged individual fibers and generally being readily apparent in routine hematoxylin and eosin (H&E)-stained sections (Dubowitz 1995; Graham and Lantos 2002) (Fig. 28.8). Where histochemical preparations are available, the enlarged fibers generally have the characteristic of type I fibers (Graham and Lantos 2002). In the early stages of the disease, occasional patients with infantile SMA have a prepathological stage where the muscle biopsy shows the features of congenital fiber-type disproportion. Accordingly, the pathologist must be extremely careful in making a diagnosis of congenital fiber-type disproportion where there is a clinical suspicion of infantile SMA.

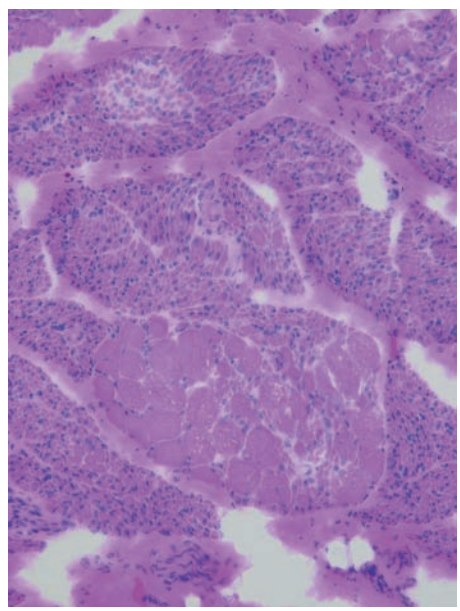

Figure 28.8. Group atrophy in a case of infantile spinal muscular atrophy (frozen section, original magnification ×200).

A number of patients with a severe form of SMA presenting with arthrogryposis and having a typical muscle biopsy appearance but negative molecular studies have been described in the literature (Kizilates et al. 2005), and we have seen similar cases in our department.

Congenital Myotonic Dystrophy

The myotonic dystrophies are subclassified as myotonic dystrophy type 1 (also known as Steinert's disease or DM1; OMIM 160900) and myotonic dystrophy type 2 (also known as Ricker syndrome, DM2, or proximal myotonic dystrophy; OMIM 602668). DM2 is a disorder presenting in adulthood and is not further described here.

Myotonic dystrophy type 1 (DM1) is a multisystem autosomal dominant disorder caused by expansion of a CTG repeat in the gene *DMPK* on chromosome 19q13.3. In normal individuals there are between 5 and 50 copies of the CTG repeat. Mildly affected individuals have 50 to 99 CTG repeats, whereas severely affected cases have 100 to 2000 or more copies (Darras 1997). The disorder shows the phenomenon of anticipation, by which the disease has an earlier presentation and more severe phenotype in successive generations. The disorder, therefore, may present congenitally, in childhood, in adulthood, as a late-onset disease, or may be asymptomatic. These clinical categories represent a continuum characterized at a molecular level by ever-increasing CTG repeat numbers in the more severe forms. In adults, symptoms and signs include weakness, myotonia, cataracts, frontal balding, cardiac conduction defects, and hypogonadism. The congenital form of DM1 has a severe phenotype and may present antenatally with reduced fetal movements and polyhydramnios. There is generalized weakness at birth, hypotonia, poor sucking, and respiratory insufficiency. Affected infants may have contractures and have a typical tented upper lip, resulting in a carp-like mouth (Machuca-Tzili et al. 2005). Early death may occur, but most survive. Survivors subsequently show delayed motor and mental development and eventually show the adult manifestations of the disease. Interestingly, the congenital form of DM1 is always maternally inherited, with the mother usually having a mild or subclinical form of the disease (Dubowitz 1995). It has been suggested that this may be explained by a negative selection biased toward the male gametes carrying large CTG expansions whereby they are less viable than oocytes carrying the mutation (Brunner et al. 1993).

Clinical investigations may be suggestive but not diagnostic of myotonic dystrophy. In adults, the EMG findings include myotonic discharges, although these may not be present in all cases. Muscle biopsy findings early in the disease include abnormally small type I fibers, with type II fibers being abnormally large. In more severe cases, features include multiple internal nuclei, sarcoplasmic masses, and an increased incidence of ring fibers superimposed on a biopsy with myopathic features including fiber necrosis, regeneration, and fibrosis (Graham and Lantos 2002). In infants, diagnosis may be difficult on electromyographic and pathological grounds as the EMG does not show myotonic discharges, and the muscle biopsy may be relatively normal, other than for atrophy or grouping of type I fibers. Farkas et al. (1974) described a distinctive "halo" surrounding the periphery of muscle fibers in transverse section stained for oxidative enzymes with this feature visible on ultrastructural examination where reduced numbers of mitochondria may also be apparent.

Definitive diagnosis of DM1 requires DNA analysis for the typical CTG repeats. Prenatal testing is available by means of DNA extracted from fetal cells obtained by amniocentesis performed at 15 to 18 weeks' gestation or by chorionic villus sampling at 10 to 12 weeks' gestation. The presence of an expanded *DMPK* allele in an affected family member should be confirmed. Abnormal test results do not predict the age of onset or the severity of the disease. However, CTG lengths of 730 to 1000 or higher are said to be more likely to be associated with congenital DM1 (Redman et al. 1993).

Congenital Myopathies

This group of conditions is often also known as the congenital ultrastructural myopathies and includes nemaline myopathy, the X-linked form

of myotubular myopathy, central core disease, minicore disease, congenital fiber-type disproportion, and minimal change myopathy.

Nemaline Myopathy

This is a congenital myopathy characterized by weakness and hypotonia with depressed or absent deep tendon reflexes. Autosomal dominant and autosomal recessive forms are described. In the mild forms of the disease, which can present between birth and adolescence, hypotonia is associated with a nonprogressive or slowly progressive muscle weakness of variable severity (Riggs et al. 2003).

The severe congenital (neonatal) form is characterized by marked muscle hypotonia and weakness, respiratory distress, and swallowing difficulties in the neonatal period (Riggs et al. 2003). Affected children often present with joint contractures and die in the first year of life. Patients affected by severe neonatal nemaline myopathy are likely to have an autosomal recessive form of the disease. Presentation in the fetal period with features of the fetal akinesia sequence has been described (Lammens et al. 1997).

The entity shows clinical and genetic heterogeneity, with mutations in five genes being identified to date: *ACTA1, NEB, TPM2, TPM3*, and *TNNT1*. These genes encode components of the sarcomeric thin filaments (Jungbluth et al. 2003).

The entity is defined by the presence of tiny rod-like structures generally visible by light microscopy in histochemical preparations stained by the modified Gomori trichrome method, but best seen under the electron microscope, in a varying number of muscle fibers (Figs. 28.9 and 28.10). The rods are composed predominantly of α-actinin (Jungbluth et al. 2003). The biopsy findings are similar irrespective of the severity of the clinical manifestations.

Myotubular Myopathy

Myotubular myopathy was first described in 1966 as the persistence of fetal muscle (Spiro et al. 1966). The entity is characterized by increased numbers of centrally located nuclei within the myofibers (Fig. 28.11). The central nuclei are often best demonstrated in longitudinal sections of muscle fibers. The nuclei are separated by areas

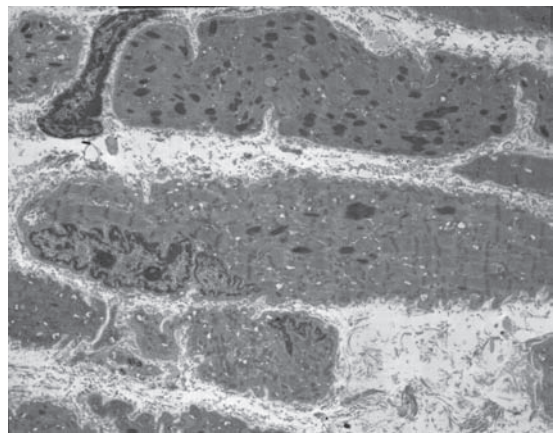

Figure 28.9. Nemaline myopathy. Note the electron-dense nemaline rods (electron micrograph, original magnification ×9000).

devoid of myofibrils but rich in mitochondria and glycogen. There is considerable clinical and genetic heterogeneity, with autosomal dominant, autosomal recessive, and X-linked forms being described. The autosomal forms generally have a later age of onset.

The X-linked form of the disease is characterized by muscle weakness that varies from mild to severe, and affected males usually present in the newborn period. The severe form often results in death in the first few weeks of life. This disease is

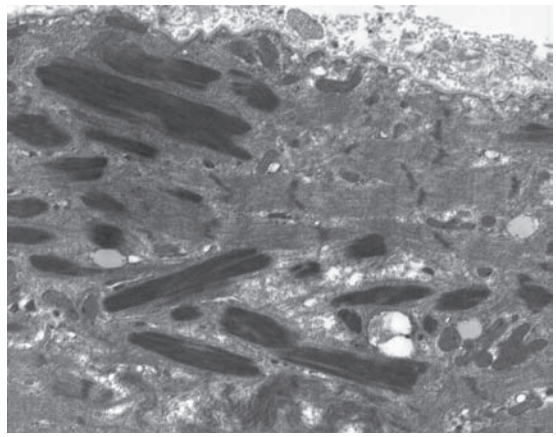

Figure 28.10. Nemaline myopathy. Electron micrograph showing morphology of the nemaline rods (original magnification ×30,000).

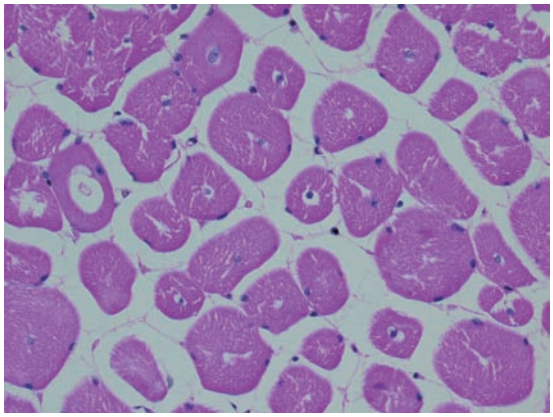

Figure 28.11. Myotubular myopathy. Note the central myocyte nuclei with surrounding halos in many of the fibers shown (original magnification ×400).

caused by mutations in the *MTM1* gene located at Xq28 and encoding for the protein myotubularin (Jungbluth et al. 2003). Genotype-phenotype correlation studies are hampered by the fact that greater than two thirds of mutations identified so far are unique and often are limited to individual families (Jungbluth et al. 2003). Intrafamilial variability is documented (Wallgren-Pettersson et al. 1995) and, recently, survival into adult life has been described (Yu et al. 2003). Diagnosis is usually by muscle biopsy, with molecular genetic testing used for confirmation, carrier detection, and antenatal diagnosis.

Other Congenital Myopathies

Congenital fiber type disproportion corresponds to a purely histologic definition where the type I fibers are significantly smaller than the type II fibers by more than 12% of the diameter of the type II fibers (Brooke 1973). Those affected generally present as floppy infants, at or shortly after birth, but many have associated contractures of the hands and feet and some also have congenital dislocation of the hip (Jungbluth et al. 2003). The clinical picture is extremely variable, with some infants having severe weakness, while others have only mild symptoms. Care should be taken in assigning this diagnosis in the first 6 months of life where the prepathological stage of infantile spinal muscular atrophy (Werdnig-Hoffmann disease) may show the statistical features of fiber-type disproportion.

Other congenital myopathies, such as central core disease (Shy and Magee 1956), multicore disease (Engel et al. 1971), and minimal change myopathy, are often not symptomatic in the fetal or neonatal period. More detailed information can be obtained in standard texts (Dubowitz 1995).

Congenital Muscular Dystrophies

These clinically and genetically heterogeneous disorders show muscle biopsy features often seen in the muscular dystrophies of later onset but have no uniquely distinguishing morphological features. This is in contrast to the congenital myopathies, each of which possesses specific and unique diagnostic features (particularly ultrastructural abnormalities) on muscle biopsy. The congenital muscular dystrophies (CMDs) may present in the neonatal period with weakness, hypotonia, joint contractures, and elevated serum creatinine phosphokinase levels, but arthrogryposis is unusual. These disorders have been previously classified on morphological grounds into two major groups depending on the association with structural brain abnormalities on neuroimaging or autopsy examination. The classification of these disorders has now been further refined following major molecular genetic and biochemical discoveries in the last decade. The disorders are now classified on the basis of the causative gene mutations and immunolocalization of proteins in affected tissues. Several of the proteins are associated with the sarcolemma and are involved in the interaction between the muscle cell and the extracellular matrix. These proteins include cell surface receptors (such as the integrins), basal lamina proteins (such as laminin-α2, also known as merosin), and extracellular matrix proteins (such as collagen VI). The other protein group includes those with known or suspected enzyme activity that act intracellularly (such as POMT1 and POMGnT1). Some of the proteins have functions yet to be determined, including fukutin, fukutin-related protein (FKRP), LARGE (putative glycosytransferase), and selenoprotein-1 (SEPN1). Diagnosis in individual cases has conse-

quently become more complex and depends on clinical features and identification of the underlying genetic and biochemical defects.

Congenital Muscular Dystrophy with Laminin-α2 Deficiency: Merosin-Deficient CMD, MDC1A

This disorder is due to mutations in the *LAMA2* gene on chromosome 6q2 with primary deficiency of laminin-α2 (Muntoni and Voit 2004). Laminins are essential components of basement membranes and, in addition, to skeletal muscle are found in the Schwann cell basement membrane and at the neuromuscular junction and myotendinous junctions. Most mutations in the *LAMA2* gene result in complete absence of laminin-α2, with a severe phenotype, but rare allelic mutations may result in partial loss of the protein with a mild or severe clinical picture depending on the effect of the specific mutation.

Affected cases present at birth or in the first few months of life with weakness (proximal muscles greater than distal with severe involvement of axial muscles), hypotonia, and respiratory and feeding problems. Contractures may occur but arthrogryposis is rare. Intelligence is usually normal. Laminin-α2 is also found in the basement membranes of brain blood vessels, oligodendrocyte tracts, and glia-limitans so that brain abnormalities may occur, with MRI studies showing demyelinating white matter changes from about 6 months of age. Structural brain changes have been reported in some patients (manifesting as polymicrogyria and hypoplasia of the cerebellum and pons) (Sunada et al. 1995). Laminin-α2 is also present in the Schwann cell basement membrane (Jimenez-Mallebrera et al. 2005) so that affected children also often have a demyelinating peripheral neuropathy.

The muscle pathology is that of a typical dystrophic process with massive fiber necrosis and regeneration combined with endomyseal and perimyseal fibrosis detectable from birth. There may be a prominent inflammatory cell infiltrate that may lead to confusion with congenital inflammatory myopathy. The diagnosis is confirmed by negative immunohistochemical staining for laminin-α2 (merosin). It is important to note that similar patterns of laminin-α2 expression may be found in Fukuyama muscular dystrophy, Walker-Warburg syndrome, and muscle-eye-brain disease, so it is important to integrate neurological imaging findings and molecular data into the diagnostic approach.

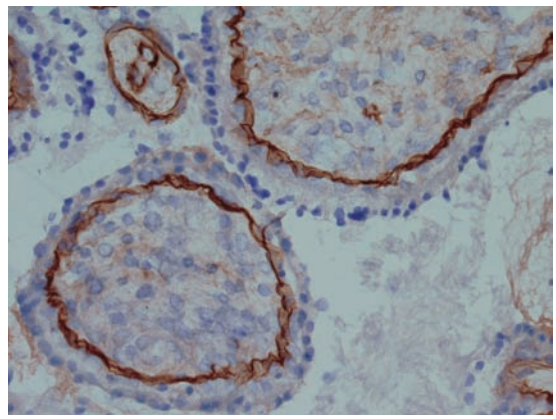

Figure 28.12. Normal chorionic villus at approximately 9 weeks gestation showing positive immunohistochemical staining of the trophoblast basement membrane for merosin (original magnification ×400).

Laminin-α2 is also expressed at the dermoepidermal junction in skin biopsies (Sunada et al. 1995; Jimenez-Mallebrera et al. 2005), and this can be used for diagnostic purposes, although it should be recognized that laminin-α2 expression in skin and muscle may differ in the same patient so that it is advisable to examine both tissues if possible. The protein is also found in the basement membrane underneath the trophoblast of chorionic villi (Fig. 28.12), with the potential for prenatal diagnosis from chorionic villus sampling (Jimenez-Mallebrera et al. 2005).

The α-Dystroglycanopathies (Including Fukuyama Muscular Dystrophy, Muscle-Eye-Brain Disease, Walker-Warburg Syndrome, MDC1C, and MDC1D)

The dystroglycan gene *DAG1* encodes for two glycoproteins known as α- and β-dystroglycan, which are associated in the cell surface binding to ligands in the extracellular matrix. The dystroglycan complex is a link between the extracellular matrix and cytoskeleton. Congenital muscular dystrophies in this group are thought to be due to

interference of the glycosylation process of α-dystroglycan, which is necessary to confer functional viability to the protein. The primary gene defects for these disorders lie in *Fukutin* (Fukuyama muscular dystrophy), *POMGnT1* (muscle-eye-brain disease), *POMT1* (Walker-Warburg syndrome), *FKRP* (MDC1C), and *LARGE* (MDC1D). These genes have a wide tissue distribution including skeletal muscle, brain, cardiac muscle, and eye (in the case of *Fukutin* and *POMT1*).

Although previously described as defined individual entities, recent molecular and biochemical data suggest that these disorders actually represent a continuous spectrum, the phenotype in each case determined by the specific mutation in each of the genes affected (Jimenez-Mallebrera et al. 2005). In general terms, Walker-Warburg syndrome is the most severe variant and MDC1C the least severe, with Fukuyama muscular dystrophy, muscle-eye-brain disease, and MDC1D intermediate in severity.

Structural brain abnormalities are common in this group. The most characteristic abnormality is type II (cobblestone) lissencephaly caused by neurons over-migrating through the glia limitans during development of cortical layering. Other abnormalities include a flattened brainstem, dilated cerebral ventricles, and delayed myelination.

Cardiac involvement with a dilated cardiomyopathy is invariable in Fukuyama muscular dystrophy and in MDC1C. Severe ocular abnormalities with myopia, cataracts, and retinal detachment are present in Walker-Warburg syndrome and muscle-eye-brain disease but are seen more rarely in Fukuyama muscular dystrophy and MDC1C.

These disorders on muscle biopsy show features of muscular dystrophy with muscle fiber necrosis and fibrosis. There is reduction in α-dystroglycan immunohistochemical staining with normal β-dystroglycan (contrasting with the reduction in both in the dystrophinopathies). The extent of reduction in staining for α-dystroglycan varies among the disorders in this group, broadly correlating with disease severity (for example completely absent in Walker-Warburg syndrome). It is important to note that these disorders also show a reduction in staining for laminin-α2 (merosin) but it is never completely absent as in primary merosin deficiency.

Prenatal genetic diagnosis is possible in families where the primary gene defect has been identified, and in this context, prenatal ultrasound scans are useful.

Ullrich Congenital Muscular Dystrophy (and the Role of Collagen VI)

Collagen VI is an extracellular matrix protein with three α chains encoded by the genes *COL6A1* and *COL6A2* (on chromosome 21q22.3) and *COL6A3* (on chromosome 2q37) (Jimenez-Mallebrera et al. 2005). Collagen VI is found in most connective tissues. It is localized to the basement membrane around each muscle fiber—perimysium and endomysium in cardiac and skeletal muscle. It is also found in Schwann cells, endoneurial cells, and perineurial cells.

Mutations in the *COL6* genes are responsible for Ullrich congenital muscular dystrophy. This disorder presents neonatally with weakness, kyphosis, contractures, torticollis, hip dislocation, and hyperextensibility of the distal joints. CK levels are usually normal or only slightly elevated. A constant feature is roughened skin due to follicular hyperkeratosis. Although collagen VI is expressed in cardiac muscle, heart involvement is not recognized as part of Ullrich congenital muscular dystrophy. This disorder is the second most common congenital muscular dystrophy after merosin-deficient congenital muscular dystrophy (MDC1A) in the West and Fukuyama muscular dystrophy in Japan.

The muscle biopsy findings include features of muscular dystrophy with variation in fiber size, necrosis and regeneration, increased perimyseal and endomyseal connective tissue, and a dramatic increase in intramuscular adipose tissue. Immunohistochemical staining for collagen VI may show complete absence from basement membrane, endomyseal, and perimyseal connective tissue. However, the appearances may be subtle with only absence at the basal lamina but normal labeling of the connective tissue. Normal or near-normal staining with collagen VI does not exclude Ullrich congenital muscular dystrophy.

In the skin, collagen VI is found in connective tissue and the basement membranes of glands, hair follicles, blood vessels, peripheral nerves, and erector pili muscles. In some cases, collagen VI

staining in skin biopsies may be completely absent, while in others it may be only slightly reduced or even normal. Collagen VI is abundant in the mesoderm within placental villi, and therefore chorionic villus samples may be utilized in prenatal diagnosis by immunohistochemistry, along with haplotype analysis.

Other Congenital Muscular Dystrophies

Several other extremely rare congenital muscular dystrophies have been described, including rigid spine syndrome (RSMD1) and congenital muscular dystrophy due to abnormalities in integrin-α7, with their own specific genetic and protein defects, but these dystrophies are not further discussed here due to their rarity and infrequent presentation in the fetal or neonatal period.

Neuromuscular Transmission Disorders

Transient Neonatal Myasthenia

In 10% to 15% of babies born to women with myasthenia gravis, there is transplacental transfer of circulating antiacetylcholine receptor antibodies (Darras 1997). The syndrome presents neonatally with weakness, feeding difficulties, respiratory difficulties, weak cry, facial diplegia, and ptosis. Arthrogryposis is rare but has been reported as part of a recurrent Pena-Shokeir phenotype [with additional features of pulmonary hypoplasia, intrauterine growth restriction (IUGR), and facial anomalies] in infants of a woman with asymptomatic myasthenia found to have antiacetylcholine receptor antibodies (Brueton et al. 2000). The severity correlates poorly with maternal severity but does correlate with maternal antibody titer. The average duration of symptoms in affected neonates is 18 days (range of 5 days to 2 months). The diagnosis is made by detecting a high serum acetylcholine receptor antibody concentration in the newborn and by rapid symptom reversal with edrophonium chloride (Tensilon).

Congenital Myasthenic Syndromes

Congenital myasthenic syndromes (CMSs) are defined as inherited disorders of neuromuscular transmission with negative testing for circulating antiacetylcholine receptor antibodies (Engel 2001). They are classified based on the site of the defect (presynaptic, synaptic, or postsynaptic). Presynaptic defects include CMS with episodic apnea (due to defective acetylcholine synthesis/packaging), paucity of synaptic vesicles, and Lambert-Eaton syndrome-like. Synaptic defects include end-plate acetylcholinesterase deficiency. The postsynaptic defects include those with decreased response to acetylcholine (slow and fast channel syndromes as well as syndromes with primary acetylcholine receptor deficiency). Diagnosis in these disorders involves assessment of clinical features, family history, EMG findings, response to acetylcholinesterase inhibitors, motor point muscle biopsies, and mutational studies.

Although present at birth, these disorders rarely present neonatally and typically present in infancy or early childhood. A comprehensive review of the approach to these disorders is covered in the 73rd ENMC (European Neuromuscular Centre) International Workshop on Congenital Myasthenic Syndromes (Engel 2001).

The Metabolic Myopathies

Although primary metabolic myopathies are inherited and are present from birth, a number of these disorders are typically diagnosed in childhood, adolescence, or adulthood. However, several disorders may present in the neonatal period. These disorders may be classified as glycogenoses, disorders of fatty acid oxidation, and the mitochondrial myopathies.

Myopathic Glycogenoses

Acid Maltase Deficiency: Pompe's Disease

Acid maltase deficiency (glycogenosis type II) manifests as two major syndromes. The first is a severe infantile generalized disease, also known as Pompe's disease, and the second (not further described here) is a milder neuromuscular disorder affecting children or adults.

Pompe's disease (OMIM 232300) presents with weakness and hypotonia in the first few weeks or months of life. Other manifestations include

macroglossia, massive cardiomegaly, and moderate hepatomegaly. Death due to cardiac failure usually occurs before the age of 2 years. Serum creatinine phosphokinase (CPK) is increased and the EMG is myopathic (Tein 1999). The electrocardiogram shows giant QRS complexes and signs of biventricular hypertrophy. Muscle biopsy shows a vacuolar myopathy with fibers containing multiple vacuoles, often coalescing into a "lacework" pattern and containing periodic acid-Schiff (PAS)-positive diastase sensitive material that stains for acid phosphatase. Electron microscopy shows excessive glycogen within the lysosomal vacuoles and free within the cytoplasm. Glycogen accumulates in other tissues, particularly the heart (Tein 1999). There is also involvement of the spinal cord anterior horn cells and brainstem nuclei, and glycogen within Schwann cells is observed on peripheral nerve biopsy.

The gene for Pompe's disease has been localized to chromosome 17 (17q25.2-q25.3). Prenatal diagnosis is possible by measuring acid maltase activity in cultured amniocytes.

Other Glycogenoses

Other disorders in this group (such as phosphorylase b kinase deficiency, phosphorylase deficiency, phosphofructokinase deficiency, phosphoglycerate kinase deficiency, and branching enzyme deficiency) typically present in older children or in adulthood. As they are rarely encountered in the fetal or neonatal period, they are not further described here.

Disorders of Fatty Acid Oxidation

Disorders of fatty acid oxidation as a group represent the most common inborn errors of metabolism and are potentially rapidly fatal. All of these disorders are autosomal recessive in inheritance. This group includes the carnitine deficiency syndromes, defects in carnitine palmitoyltransferases (CPT I and II), carnitine-acylcarnitine translocase deficiency, very-long-chain acyl–coenzyme A (CoA) dehydrogenase deficiency (VLCAD), trifunctional protein deficiency, short chain acyl-CoA deficiency (SCAD), and short chain hydroxyacyl-CoA dehydrogenase deficiency (SCHAD). These disorders most commonly present in infants, children, and adults, and are rarely seen in the neonatal period. Muscle biopsy findings may include lipid accumulation, mainly in type I fibers. The diagnosis of the precise underlying defect requires detection of enzyme deficiency in cultured skin fibroblasts or muscle tissue. The reader is referred to two comprehensive reviews for further in-depth description of these disorders (Tein 1999; Vladutiu 2000).

Mitochondrial Myopathies

These are rare inherited disorders of mitochondrial energy metabolism present in approximately 1 in 10,000 live births. Muscle biopsy is sensitive in detecting mitochondrial disease but lacks the specificity of a genetic diagnosis. Genetic screening, however, detects only a small percentage of patients with mitochondrial disease, so the pathologist still has a role to play in identifying patients affected by these disorders. The clinical and genetic heterogeneity of these disorders makes their investigation and diagnosis a challenge. A combination of techniques, including muscle histochemistry, biochemical respiratory chain assessment, and molecular genetic studies, is required. The majority of these disorders are not usually encountered in the neonatal period.

Cytochrome c Oxidase Deficiency

Fatal and benign forms of this disorder may present in neonates. The fatal form manifests as severe lactic acidosis, profound hypotonia with weakness, and feeding and respiratory difficulties. The majority of affected infants die before 1 year of age with cardiorespiratory failure. Muscle biopsy shows ragged-red fibers with glycogen and lipid accumulation in most cases. On ultrastructural examination, these fibers show predominantly subsarcolemmal accumulation of mitochondria, seen also as subsarcolemmal activity for NADH dehydrogenase on histochemical staining (Figs. 28.13 to 28.15) (Dubowitz 1995). Cytochrome oxidase histochemical staining shows virtually complete absence of activity.

The benign form of cytochrome oxidase deficiency presents with generalized weakness and

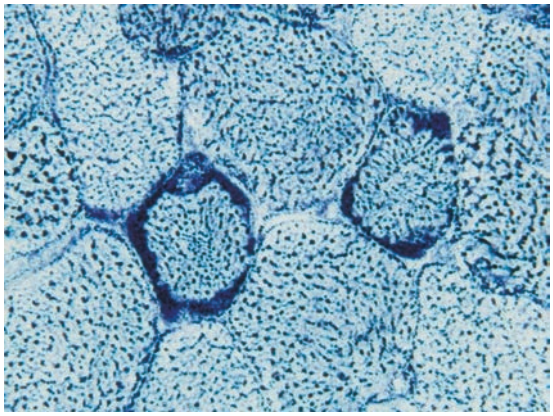

Figure 28.13. Mitochondrial myopathy. Note accentuation of staining in subsarcolemmal region of two myofibers on NADH histochemical staining (original magnification ×600).

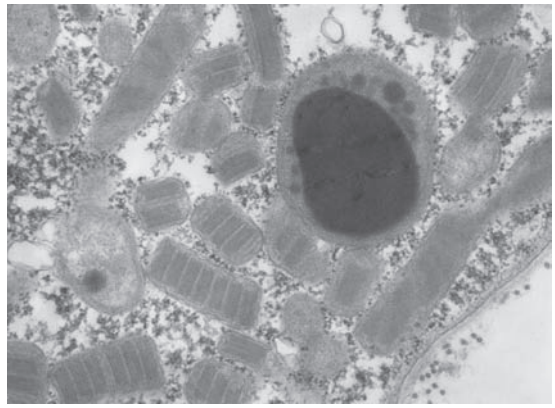

Figure 28.15. Mitochondrial myopathy. Electron micrograph showing morphology of abnormal mitochondria with radiator core and lipid inclusions (original magnification ×60,000).

hypotonia, respiratory and feeding problems, and severe lactic acidosis. The disorder is localized to skeletal muscle in its involvement, unlike the fatal form of the disease. Clinically, affected infants usually improve in the first year of age and are normal by age 3. As in the fatal form, the muscle biopsy shows ragged-red fibers and undetectable cytochrome oxidase activity on histochemical staining. In contrast to the fatal form, cytochrome oxidase is detected on enzyme-linked immunosorbent assay (ELISA) immunological analysis of muscle tissue (Darras 1997).

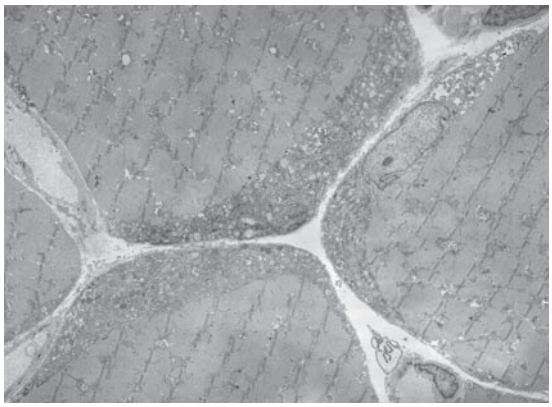

Figure 28.14. Mitochondrial myopathy. Electron micrograph (original magnification ×4800) showing subsarcolemmal accumulation of mitochondria.

Peripheral Neuropathies

Acquired peripheral neuropathies are uncommonly encountered in the neonatal period and are essentially confined to brachial plexus injuries secondary to birth trauma, with wallerian degeneration of the nerve distal to the lesion.

Inherited peripheral neuropathies are common and are responsible for a significant proportion of neuropathies, but they are rarely encountered in the fetal or neonatal period, usually presenting later in childhood or adulthood. The ability to measure nerve conduction velocities provided the basis for the classification scheme proposed by Dyck (2002). This resulted in three classification groupings: (1) hereditary motor and sensory neuropathies (HMSN); (2) hereditary sensory and autonomic neuropathies (HSAN); and (3) hereditary motor neuropathies (HMN). However, classification and diagnosis has become much more complex due to the rapid emergence of new information regarding the underlying genetic defects causing these disorders. This has resulted in the expansion of the above classification to include multiple subgroupings. Genetic testing is based on an approach incorporating clinical assessment of the presence of associated neurologic (brain, muscle, and spinal cord) or nonneurological systems, mode of inheritance, and nerve conduction studies (demyelinating or axonal). Genetic tests are available for a limited number of these

disorders but are expected to increase in number in the future.

Inherited Motor and Sensory Neuropathy

The classification within this group of disorders (also known as Charcot-Marie-Tooth disease or the peroneal muscular atrophies) has become highly complex as the number of causative genetically distinct entities has risen dramatically in the last decade. There are now seven subgroups (HMSN I to HMSN VII), which share many clinical features including distal leg weakness, distal symmetrical sensory loss, pes cavus, and reduced or absent deep tendon reflexes. HMSN type III is the subgroup most likely to present in the neonatal period or early infancy.

Hereditary Motor and Sensory Neuropathy Type III (Dejerine-Sottas Syndrome, HMSN type III)

This subgroup was originally described by Dejerine and Sottas in 1893. A similar phenotype results from different mutations, and the features are those of proximal limb and trunk weakness with areflexia and, in time, hypertrophic nerves. Cerebrospinal fluid (CSF) protein levels are markedly raised. Patients have extremely low nerve conduction velocities (less than 10 m/s) and sural nerve biopsy shows profound demyelination with prominent onion bulb formation (Scott and Kothari 2005).

HMSN type III is most commonly sporadic, but autosomal recessive and dominant forms have been described (Klein and Dyck 2005; Scott and Kothari 2005). There are four subtypes (IIIA to IIID) and each has its own characteristic genetic abnormality: IIIA is linked to mutations in the *PMP22* gene on chromosome 17p, IIIB is linked to mutations in the *PO* gene on chromosome 1q, IIIC to mutations in the *EGR2* gene on chromosome 10q, and IIID has been mapped to chromosome 8q23 (Klein and Dyck 2005; Scott and Kothari 2005).

Hereditary Sensory and Autonomic Neuropathy

The hereditary sensory and autonomic neuropathies (HSANs) are a group of familial neuropathies with involvement of sensory and autonomic nerves. There are five subtypes and as a group they are rare compared with HMSN. HSAN III is the subtype most likely to present in the neonatal period (Scott and Kothari 2005).

Hereditary Sensory and Autonomic Neuropathy Type III

Hereditary sensory and autonomic neuropathy type III (HSAN III) (also known as Riley-Day syndrome) presents with low birth weight, failure to thrive, feeding difficulties, and autonomic dysfunction (including alacrima, poor temperature regulation, hypertension, and hyperhidrosis) (Scott and Kothari 2005). Later in the disease, sensitivity is diffusely impaired. Unlike other subtypes of HSAN, motor nerves are also involved with slow conduction velocities. Nerve biopsy shows severe loss of peripheral unmyelinated and myelinated fibers smaller than 12 μm in diameter, in addition to loss of neurons in the dorsal root and autonomic ganglia. This autosomal recessive disorder presents almost exclusively in Ashkenazi Jews and results from mutations in the *IKBKAP* gene on chromosome 9q31 (Ryan and Ouvrier 2005; Scott and Kothari 2005).

Other Inherited Peripheral Neuropathies

These neuropathies are all unlikely to be encountered in the neonatal period and include the hereditary distal motor neuropathies, spinocerebellar ataxias, and the hereditary spastic paraplegias with neuropathy. All have multiple causative genetic defects and variable patterns of inheritance.

The multiple system-inherited neuropathies affect multiple systems, both neural and nonneural. They are typically caused by inborn errors of metabolism with specific enzymatic defects and include such disorders as Fabry's disease, transthyretin amyloidosis, the leukodystrophies, and giant axonal neuropathy. They rarely present in the neonatal period. Diagnosis is based on metabolic assays with or without associated genetic testing, precluding the need for nerve biopsy in most cases (Klein 2004).

Prenatal Diagnosis of Neuromuscular Disease

Fetal Ultrasound Examination

Ultrasound is used routinely in the first trimester to estimate fetal age and to identify features such as nuchal fold thickness, which may indicate increased risk of chromosomal disorders. In the second trimester, fetal ultrasound enables morphological assessment of fetal anatomy and development. Features that may suggest a neuromuscular disorder include lack of fetal movements, hydrops, and polyhydramnios. Polyhydramnios associated with absence of a visible stomach may indicate the lack of fetal swallowing movements. Contractures may be visible along with abnormalities of posture such as talipes. In contrast, contractures (specifically talipes) associated with oligohydramnios should prompt careful examination of the genitourinary tract including the kidneys (for agenesis or multicystic change) and the bladder (for signs of bladder outlet obstruction).

Second-trimester ultrasound is particularly useful in detecting disorders resulting in impaired neuromuscular development early in gestation (such as arthrogryposis and its various underlying disorders). When suggestive features are identified, further investigative techniques (such as amniocentesis or chorionic villus sampling to facilitate karyotyping, genetic testing for specific neuromuscular disorders, and infection screening) can be planned. Further imaging such as MRI scanning may be required to enable greater visualization of the brain and identification of coexisting cerebral or cerebellar malformations. These additional investigations in the second trimester provide further information, forming the basis for parental counseling. A decision may then be made to terminate the pregnancy or make appropriate plans for delivery and neonatal management if it is elected to proceed with the pregnancy.

Ultrasound does not detect many of the conditions resulting in neonatal hypotonia (such as infantile spinal muscular atrophy, the congenital myasthenic syndromes, and the metabolic myopathies) as these are disease processes that typically become manifest later in gestation and do not result in morphological features identifiable on second trimester imaging.

Prenatal Genetic Testing

The majority of the congenital myopathies, congenital muscular dystrophies, and inherited peripheral neuropathies as well as some of the arthrogryposis disorders have known underlying genetic abnormalities for which genetic testing is now commercially available or potentially available on a research basis. There has recently been an avalanche of information regarding the causative genetic defects in this field of disorders and, flowing from this, the ability to test for the identified genetic abnormalities.

Prenatal diagnosis is now possible utilizing material obtained by amniocentesis, chorionic villus sampling, or fetoscopy with fetal skin biopsy. Specific disorders (and the responsible genes) for which prenatal genetic is now available include infantile spinal muscular atrophy (*SMN1, SMN2*), nemaline myopathy (*ACTA1*), congenital myotonic dystrophy (*DMPK*), X-linked myotubular myopathy (*MTM1*), central core disease (*RYR1*), lethal popliteal pterygium syndrome (*IRF6*), the congenital muscular dystrophies (*FCMD* for Fukuyama muscular dystrophy, *POMGnT1* for muscle-eye-brain disease, *POMT1* for Walker-Warburg syndrome, *FKRP* for MDC1C, and *LARGE* for MDC1D), Pompe's disease (*GAA*), and the inherited peripheral neuropathies. Techniques include targeted mutation analysis, mutation scanning, and sequence analysis of the specific coding regions. Comprehensive and continually updated information regarding this ever-expanding field is available online at www.genetests.org with cross-referencing to the compendium to the Mendelian Inheritance in Man at www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM.

The decisions to be made in genetic testing are complex because multiple genetic tests are often available; testing is costly, often requiring material to be sent to other centers; and the results may be of uncertain significance. Once the differential diagnosis has been narrowed as much as possible to a category of disorders (on the basis of clinical features, pregnancy, family history, electrophysiological muscle and nerve testing, etc.), genetic testing must be coordinated by a physician in a

position to then perform the appropriate counseling, such as a clinical geneticist.

Investigation of Neuromuscular Disease

The development of molecular biological techniques and their application to the diagnosis of neuromuscular diseases, together with the increasing sophistication of prenatal ultrasound, demands that the investigation of neuromuscular diseases in the fetus and neonate be a multidisciplinary process involving a combination of the fetal ultrasonographer, neonatologist, geneticist, dysmorphologist, and metabolic physician, together with the pathologist, to decide the most appropriate methods of investigation.

It has become self-evident in the last decade that formal muscle biopsy is indicated much less often than in the past. The ability to detect certain genetic disorders on the basis of molecular analysis carried out on blood, amniotic fluid, or even by chorionic villus sampling in early pregnancy has led to a marked diminution in the numbers of muscle biopsies undertaken. This is particularly so since the commonest disorders seen in clinical practice such as Xp21 dystrophinopathy (Duchenne/Becker muscular dystrophy) and infantile spinal muscular atrophy (Werdnig-Hoffmann disease) can be diagnosed by molecular means in approximately 70% and 95% of cases, respectively. It is often the case in recent years that muscle biopsy is not undertaken until widespread testing with the less invasive molecular biological techniques has been completed.

In those patients where a diagnosis is not readily established by molecular means or in those situations where the techniques are not available, but where muscle biopsy is considered appropriate, it is important to take muscle samples for routine paraffin sections, enzyme histochemistry, and electron microscopy. At the same time it is prudent to establish a fibroblast line and to ensure that this sample is stored for future analysis as the range of molecular techniques is expanded. In such cases, in addition to routine H&E staining, the range of histochemical techniques applied should include PAS, lipid stains, techniques for mitochondrial enzymes [NADH-TR, lactate dehydrogenase (LDH), or succinate dehydrogenase (SDH)] and the modified Gomori trichrome technique.

Immunohistochemical analysis of expression of membrane-related proteins (dystrophin, sarcoglycans, and merosin) helps in the diagnosis of various forms of muscular dystrophy.

The same techniques can be applied to necropsy material obtained from fetuses or newborn infants. In such cases it is often useful to sample muscles from different topographical sites (proximal and distal). In addition, detailed examination of the brain and spinal cord is indicated to assist in determining those conditions with neurogenic origin. It may be worth considering preautopsy cranial MRI scanning if there is a suggestion on antenatal ultrasound of a cerebral or cerebellar abnormality. If a metabolic disorder is suspected (as in the case of neonatal death of a floppy infant), an approach incorporating protocols for metabolic disorders may need to be used, and the autopsy may need to be performed urgently immediately following death in order to provide appropriate samples for enzyme assays. If it is necessary to examine the spinal cord and perform formal neuropathological examination of the brain, this should be explained to the parents as part of the consent process. The case should be discussed with a clinical geneticist and metabolic physician (especially if they have been involved in the management prior to pregnancy termination or neonatal death). Specific information that should be sought includes what, if any, investigations have been performed already prior to autopsy. These may need repeating or may dictate follow-up autopsy investigations.

Autopsy Approach

Following discussion with a clinical geneticist and metabolic physician, a full neuromuscular autopsy is generally required in cases of suspected congenital neuromuscular disorders. In addition to standard autopsy examination procedures, additional techniques are required in these cases. A full skeletal survey should be performed to assess bone development. As part of the external examination, close-up photographs should be taken of facial features and contractures, and to verify the

presence or absence of pterygium formation. A thorough description of contractures is important. In cases of intrauterine constraint, contractures are often asymmetrical, whereas in primary neuromuscular disorders, they are usually symmetrical. In fetuses and neonates, muscle samples should be taken from multiple sites (including contracted and opposing muscles of the upper and lower limbs, intercostal muscles, diaphragmatic muscle, and paravertebral muscle). The distribution of the muscle abnormality may be helpful in determining the underlying disorder. Muscle tissue should be divided for snap-freezing (for enzyme assays if required), histology, and electron microscopic examination. Tissue should also be sampled for cytogenetic studies (skin biopsy, pericardium, gonad).

The brain should be examined formally following fixation with photographs of external surfaces and slices following sectioning. The spinal cord should be dissected in its entirety and sections submitted from each of the cervical, thoracic, and lumbar segments, as neuronal loss may not be uniform along the whole length of the cord.

It is generally not practical to examine peripheral nerves in fetuses, but in the neonate it may be important to dissect the sural nerve if there is clinical suspicion of an inherited neuropathy.

A section of skin should also be submitted for histology, particularly if there is any suggestion of restrictive dermopathy as a possible diagnosis (evidenced by tight skin).

When submitting a skin sample for cytogenetic studies, it is important to highlight on the request form that a fibroblast cell line should be frozen and stored permanently when karyotyping has been completed. This is because new genetic tests are becoming available on a regular basis, and even if a specific diagnosis cannot be made at the time of autopsy, further testing may be available in the future.

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29
The Skeletal System

Peter G.J. Nikkels

The different phenotypes of disturbances of skeletal development have for centuries fascinated artists as well as medical men, clearly seen in the painting of Don Sebastian de Mora by Velasquez, in the Prado in Madrid.

Skeletal dysplasias are a group of disorders with a disturbance in development or growth of cartilage or bone. Epiphysis, metaphysis, and diaphysis of long bones are affected in a generalized manner with or without involvement of the membranous bone of the skull. A dysostosis affects one or some skeletal elements while the other bones remain normal. There are approximately 200 different disorders known that involve the skeleton. Some 50 dysplasias are lethal. Lethality is usually based on thoracic underdevelopment and lung hypoplasia. The overall frequency among stillbirths and liveborns has been estimated to be 1 per 4000 to 1 per 6000 births and the frequency among perinatal deaths to be 1 per 110 deaths (Camera and Mastroacovo 1982; Orioli et al. 1986; Källén et al. 1993; Rasmussen et al. 1996).

The recognition of congenital abnormalities and the classification of syndromes in general require careful attention to detail. This is equally true for congenital skeletal dysplasias incompatible with life. Besides documentation of external abnormalities in such cases, radiographic examination of the skeleton before autopsy is essential (Winter et al. 1984; Hall 1992). Skeletal dysplasias with a disorder of bone are characterized by osteopenia or osteosclerosis, while disorders of cartilage usually give rise to a short stature due to a defective growth of long bones. The skeletal dysplasias with a cartilage disorder are usually disproportionate, with abnormal short limbs with or without a short trunk. Fetuses with a relative normal proportion have other defects such as hormone deficiencies. Measuring the length of the long bones and spine on an x-ray is helpful in determining whether it is a (disproportionate) skeletal dysplasia. For the normal values of fetal long bone growth and spine (from 13 to 40 weeks gestational age) and the correlation with ultrasound data, see van der Harten et al. (1990). Classification of skeletal dysplasias is based on the localization of the affected bony structure; see Table 29.1 for the appropriate terminology. The diagnosis is made primarily on radiological features, although the remainder of the postmortem examination is still important, and sometimes findings unrelated to the skeletal system are very helpful in making the diagnosis. Interpretation of the radiographs should be based on knowledge of morphology and the dimensions of the normal skeleton at different stages of development (van der Harten et al. 1990). Histological examination of cartilage and bone may clinch the diagnosis in several forms of dysplasia. In cases of suspected lethal bone dysplasia, the following radiographic examinations should be undertaken: anteroposterior view of the whole body (babygram); lateral view of the whole body; lateral view of the skull; and arm/hand and leg/foot, separately (Cremin and Beighton 1978; Kozlowski 1985; Lachman 1994).

To demonstrate ossification in very young fetuses more clearly, the contrast of radiographs can be increased by immersion of the whole body overnight in a 0.5% aqueous solution of silver nitrate (O'Rahilly and Meijer 1956; van Zalen-

Table 29.1. Terminology for the classification of skeletal dysplasias

Axial skeleton		Appendicular skeleton	
Skull	Cranio/cranial	Location	Epiphyseal (growth plate and joint cartilage)
Face	Facio/facial		Metaphyseal (primary and secondary spongiosum below growth plate)
Mandible	Mandibulo-		Diaphyseal (trabecular bone, mid-shaft)
Clavicle	Cleido-		
Ribs	Costo-	Shortening	Rhizomelic (proximal, e.g., femur)
Spine	Spondylo/vertebral		Mesomelic (middle, e.g., tibia/fibula)
Pelvis	Ischio/ileo/pubic		Acromelic (distal, hands/feet)

Sprock et al. 1997). Histological examination of cartilage and bone is primarily directed at the growth plate or physis and resting cartilage (Yang et al. 1976; Sillence et al. 1979a; Gilbert et al. 1987). For adequate histological examination, the head of the humerus and the femur (with the metaphysis and part of the diaphysis) are necessary, in addition to vertebral bodies, the costochondral junction of the rib, and the pharyngeal/tracheal cartilage. The severity of radiographic abnormalities of the metaphysis normally parallels the degree of disorganization at the growth plate on histological examination.

Osteochondrodysplasias

Osteochondrodysplasias are abnormalities in development or growth of cartilage or bone leading to faulty development of the appendicular or axial skeleton. The first classification of osteochondrodysplasias was made in 1969, thanks to an initiative of the European Society of Paediatric Radiology. Because of increasing interest in this field, which had led to the recognition of new types of dysplasia, this so-called Paris classification was revised several times. The classification of 1992 (Spranger 1992) was exclusively based on radiodiagnostic criteria, grouping morphologically similar disorders into 24 bone dysplasia "families" (Spranger 1988). Later classifications were based on the combination of radiology/morphology and molecular genetics (Horton 1996; McKusick et al. 1996; Rimoin 1998). The most recent classification, the International Nosology and Classification of Constitutional Disorders of Bone (2001), was expanded to 33 osteochondrodysplasia groups, and three genetically determined dysostoses were added as well. This classification is also a combination of morphology and genetics (Hall 2002). However, it is becoming clear that due to the different interests of the professionals dealing with skeletal dysplasias, two parallel classification systems are needed—one with clinical signs and symptoms, and another with a more genetics- or research-oriented basis. The latter classification, proposed by Kornak and Mundlos (2003), was based on the development of bone using embryology and molecular biology. It is based on the normal development of bone, which starts with pattern formation of the skeleton, followed by early differentiation, growth (chondrocyte differentiation and proliferation, extracellular matrix composition), and finally skeletal homeostasis.

The development of bone can be divided in three compartments. The appendicular skeleton starts with the formation of the mesenchymal anlage. The axial skeleton is somite derived, and neural crest–derived cells form the craniofacial skeleton. As soon as the pattern formation is finished during early embryogenesis, cells migrate to these areas and these cells differentiate into cartilage-forming chondrocytes in the endochondral skeleton and into bone-forming osteoblasts of the membranous skeleton (as in ossification of the skull). Most of the skeleton is formed by endochondral ossification. After formation of the cartilage anlage, it is replaced by bone. This process starts in the middle; the cartilage is mineralized and removed by osteoclasts. This is followed by ingrowths of vessels, and the periosteum produces osteoblasts to form the cortex, the primary center of ossification.

Important for linear growth and increase in diameter is the formation of the growth plate, the secondary center of ossification. This is a highly specialized cartilage structure arranged in columns of reserve, proliferating, and hypertrophic chondrocytes that are replaced by bone in the primary spongiosa (Fig. 29.1). The composition of the extracellular matrix changes considerably in this region, and this structure is responsible for linear bone growth; several signaling pathways control the chondrocyte proliferation. Some defects in these signaling pathways result in proportionate short stature and some result in disproportionate short stature, probably because the differentiation and production of extracellular matrix are also disturbed. One of these growth control peptides is the parathyroid hormone–related peptide (PTHrP). Using transgenic technology, so-called knockout mice homozygous for the PTHrP null mutation showed widespread abnormalities of enchondral bone formation, in combination with lung hypoplasia, and they consequently die postnatally from asphyxia (Karaplis et al. 1994). Histological examination revealed a diminution of chondrocyte proliferation, associated with premature maturation of chondrocytes and accelerated bone formation. These characteristics do bear resemblance to those from fetuses with "Blomstrand" osteochondrodysplasia, a rare form of lethal skeletal dysplasia (Den Hollander et al. 1997; Oostra et al. 2000). As an example of "reverse genetics," it was proven that Blomstrand dysplasia indeed results from the absence of functional PTH/PTHrP receptors (Jobert et al. 1998; Karperien et al. 1999).

The extracellular matrix is essential for the normal function of cartilage and bone. The composition of the extracellular matrix is different in the different regions. Collagen types II, IX, and XI can be found in the articular cartilage. Defects in collagen II give rise to a wide spectrum of skeletal dysplasias ranging from lethal to very mild, such as achondrogenesis II or Langer-Saldino dysplasia, Torrance dysplasia, hypochondrogenesis, spondyloepiphyseal dysplasia congenita, and Kniest and Stickler dysplasia. Collagen type X is formed by the hypertrophic chondrocytes in the epiphyseal growth zone, and collagen type I can be found in bone. Some other extracellular matrix proteins can be found in cartilage as well, such as perlecan and matrillin. Mutations in these genes result in different types of osteochondrodysplasias.

Another example of important matrix molecules are glucosaminoglycans. They are not only a major matrix component but they are also very

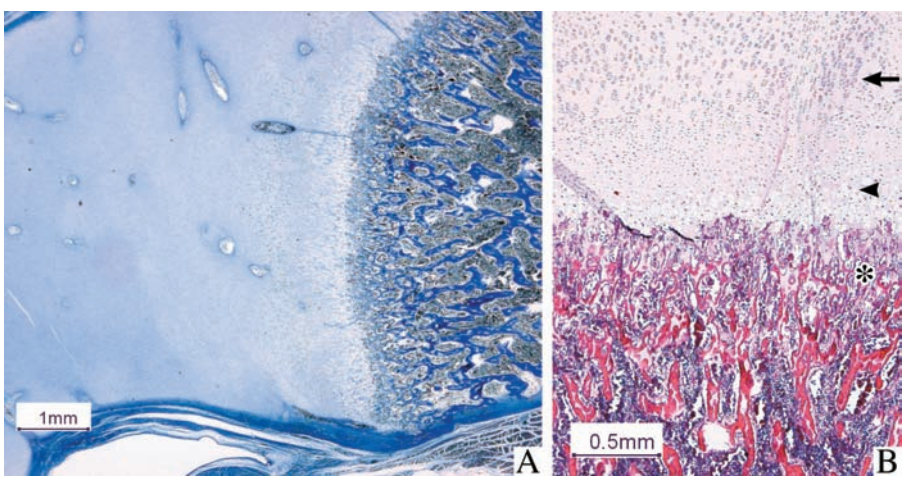


Figure 29.1. Normal histology. (A) Low-power view of normal growth plate (Ladewig, trichrome stain) from a 26-week-old normal fetus with occasional vessels in resting cartilage. (B) Medium power of the same growth plate with the resting, proliferative (arrow) and hypertrophic (arrowhead) cartilage and the primary spongiosa (asterisk) were cartilage is replaced by bone (hematoxylin and eosin, H&E).

important as a cofactor for normal growth factor signaling. Sulfation of these molecules is very important for normal functioning, and a defect in the sulfate-transporter gene, the diastrophic dysplasia sulfate transporter (DTDST) gene, also causes a wide variety of skeletal dysplasias depending on the rest of the activity of the transporter. In decreasing severity achondrogenesis type IB is followed by atelosteogenesis type II, diastrophic dysplasia, and the autosomal recessive form of multiple epiphyseal dysplasia.

The osteochondrodysplasias, which are lethal before or shortly after birth, are particularly important for the pathologist and will be considered in detail here. Figure 29.2 is a flow diagram based on typical radiological features. The most common of the rare lethal osteochondrodysplasias are mentioned in this figure with some histological features of bone and cartilage of these lethal dysplasias as well. The correct diagnosis of bone dysplasias is important because of the implications for parental counseling on the prospects for future pregnancies. Second-trimester and sometimes first-trimester ultrasound diagnosis can be confidently offered in some cases (Hobbins et al. 1982; Wladimiroff et al. 1984; Brons et al. 1990; Romero et al. 1990; Sharony et al. 1993; Goncalves and Jeanty 1994; Lachman 1994). Diagnosis may follow the birth of an affected baby or the recognition of a complication of the pregnancy such as polyhydramnios, fetal hydrops, or an inappropriately small uterine size for gestation. An increasing proportion of first affected babies are being identified during ultrasound examination in the second trimester of pregnancy for unrelated reasons (Sharony et al. 1993; Rasmussen et al. 1996). For this reason, too, pathologists may find themselves examining a fetus with a nonlethal type of dysplasia. Beighton (1988), Spranger and Maroteaux (1990), and Taybi and Lachman (1990) discuss findings in other bone dysplasias.

FGFR3-Related Lethal Skeletal Dysplasias—The Achondroplasia Group

Mutations in the fibroblast growth factor receptor 3 (FGFR3) can cause several skeletal dysplasias, and these are all autosomal dominant. The lethal types are thanatophoric dysplasia types I and II. The nonlethal SADDAN dysplasia (severe achondroplasia with developmental delay and acanthosis nigricans) and achondroplasia are also caused by mutations in the *FGFR3* gene (Shiang et al. 1994; Tavormina et al. 1995; Bellus et al. 1999). Different mutations in this gene cause the different types of these skeletal dysplasias (Cohen 2002). Thanatophoric dysplasia is the most common form of lethal skeletal dysplasia and was distinguished from classic achondroplasia by Maroteaux et al. (1967). Unlike achondroplasia this dysplasia is normally not compatible with life (hence the name, derived from the Greek *thanatos*, death, and *phorus*, seeking). The longer survival of some patients may well be due to the effect of modern neonatal care (MacDonald et al. 1989; Baker et al. 1997).

Thanatophoric Dysplasia with Bowed Femora with or Without Cloverleaf Skull (Type I)

In thanatophoric dysplasia the trunk is relatively long but the limbs are short with exo- or endorotation of the legs and feet. The head is relatively large with craniofacial disproportion. Radiographically the skeleton shows platyspondyly with U- or H-shaped vertebrae. The ribs are short, making the thorax narrow and pear- or barrel-shaped. The most striking radiographic characteristic is bowing of the shortened femur, the so-called (French) telephone receiver femur, with cupping and flaring of metaphyses. There is not much phenotypic variation of expression in thanatophoric dysplasia, not even at different gestational ages (Fig. 29.3). Histologically, the resting cartilage has normal cellular density with an abundant homogeneous matrix. Enchondral ossification is severely disturbed. Hypertrophic chondrocytes are recognizable but in disorderly arrangement. The most characteristic abnormality is hypertrophy of the periphysis with penetration of the growth plate so that fibrous disorganization with formation of plump, haphazardly arranged, bony trabeculae

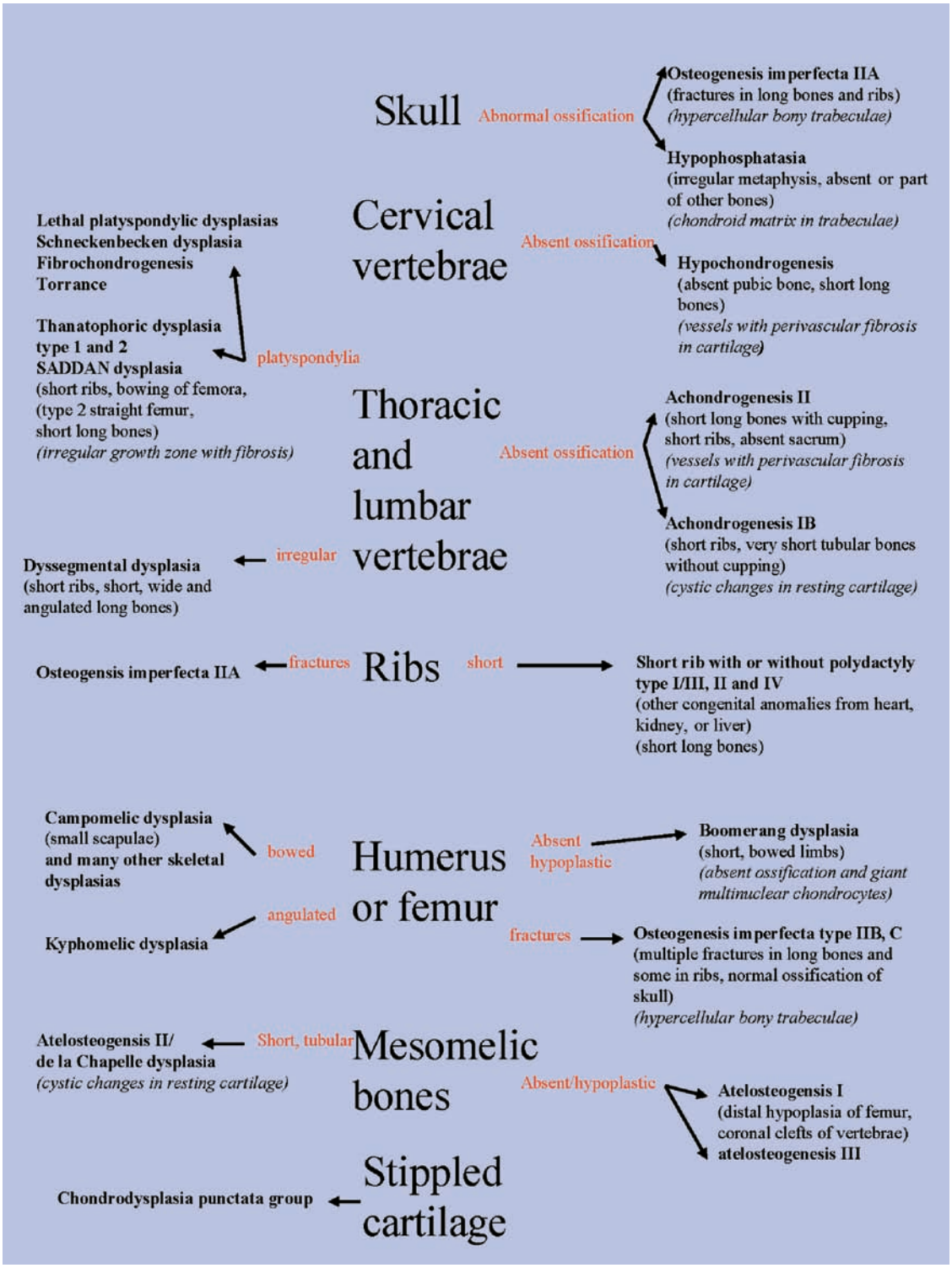


Figure 29.2. Diagram of the most commonly encountered rare lethal osteochondrodysplasias. The typical radiological abnormalities and some characteristic histological features are cited.

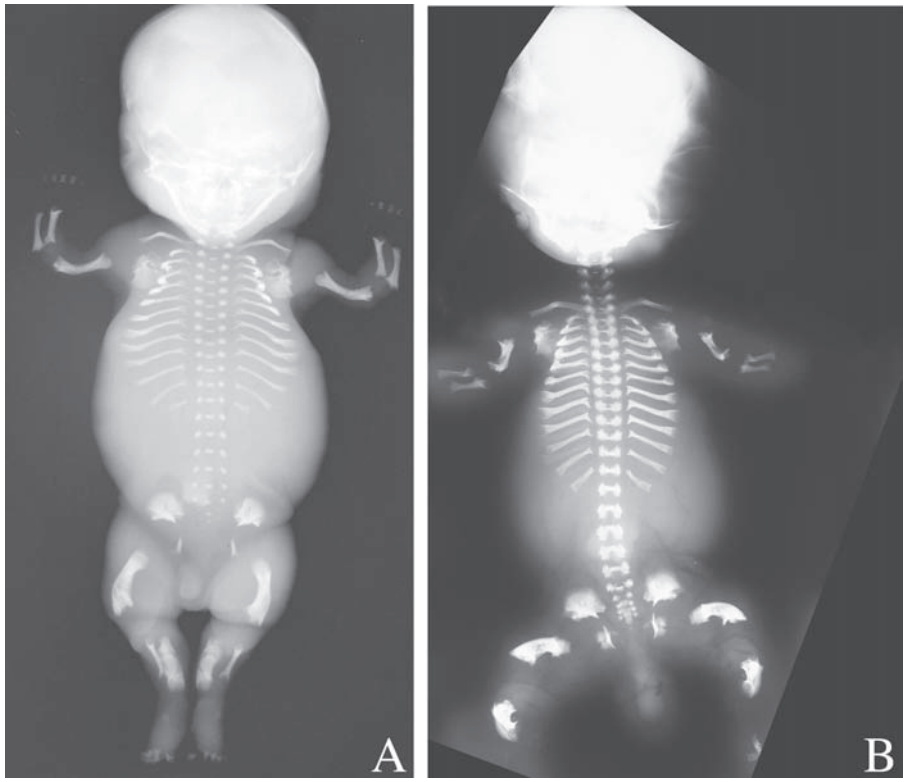


Figure 29.3. Thanatophoric dysplasia type I. Babygram from 19-week (A) and 34-week (B) fetus with short ribs, severe platyspondylia, U- and H-shaped vertebrae, and bowing of femora.

is apparent (Maroteaux et al. 1976; Ornoy et al. 1985a). This fibrous disorganization of the growth plate is not uniform (Fig. 29.4). Abnormalities in the central nervous system (CNS) have been described and mainly affect the temporal lobe (Wongmongkolrit et al. 1983; Ho et al. 1984; Knisely and Ambler 1988; van der Harten et al. 1993).

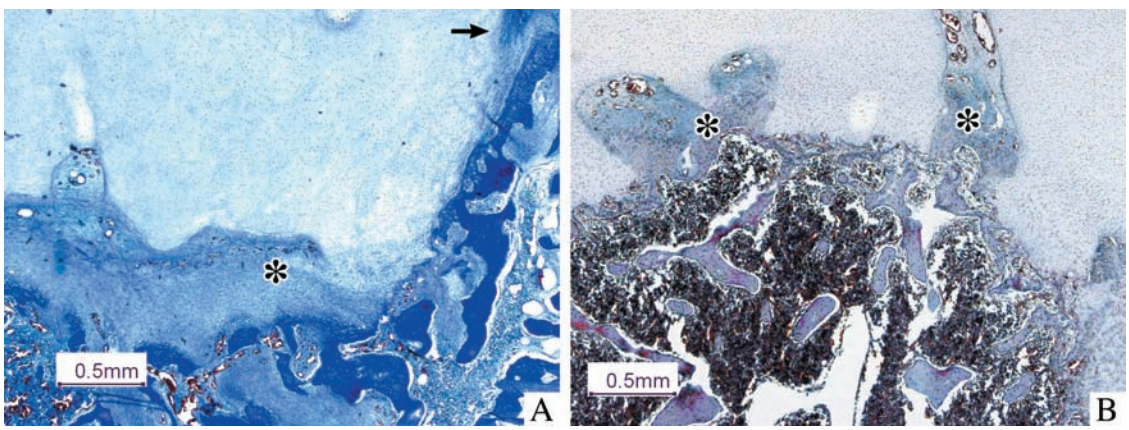


Figure 29.4. Thanatophoric dysplasia type I. (A,B) The epiphyseal growth plate of a 34 weeks' gestation fetus with an irregular transition between bone and cartilage with occasional islands of fibrotic tissue (*), increased periosteal ossification, and fibrotic tissue penetrating the growth zone from the groove of Ranvier (arrow).

Thanatophoric Dysplasia with a Straight Femur with or Without a Cloverleaf Skull (Type II)

Holtermüller and Wiedemann (1960) described a form of craniosynostosis with trilocular configuration of the skull and severe internal hydrocephalus under the term *Kleeblattschädel* (cloverleaf skull) as an isolated anomaly and in combination with a generalized skeletal dysplasia. Langer et al. (1987) described a different type of thanatophoric dysplasia that shared the phenotype with the more common type but with straight, instead of bowed, femora often in combination with a cloverleaf skull (Fig. 29.5). However, a cloverleaf skull may also accompany the common type of thanatophoric dysplasia as well. The abnormalities of the remainder of the skeleton and the histological appearance of the growth plate are comparable to those seen in thanatophoric dysplasia without craniosynostosis. The SADDAN dysplasia shows a comparable histology of the growth plate as well.

Collagen Type I Disorders; Osteogenesis Imperfecta

The second most common lethal skeletal dysplasia is osteogenesis imperfecta or brittle bone disease. Different types and subtypes have been described (Sillence et al. 1979b; Maroteaux et al. 1986). The Sillence classification has gained wide usage. It describes four categories, of which type II is the perinatal lethal type. Within type II there are three separate variants, A, B, and C, which can be distinguished by radiography. In contrast with previous publications, all well-studied cases of lethal osteogenesis imperfecta are autosomal dominant with a rare occurrence of the disorder in the next pregnancies based on germline mosaicism. The empirical recurrence risk based on this germline mosaicism in one of the parents is 7%. However, there are some other skeletal dysplasias with autosomal recessive inheritance that show some resemblance to osteogenesis imperfecta; several of these skeletal dysplasias are not lethal, such as Bruck, osteoporosis-pseudoglioma, and Cole-Carpenter syndromes (Cole 2002).

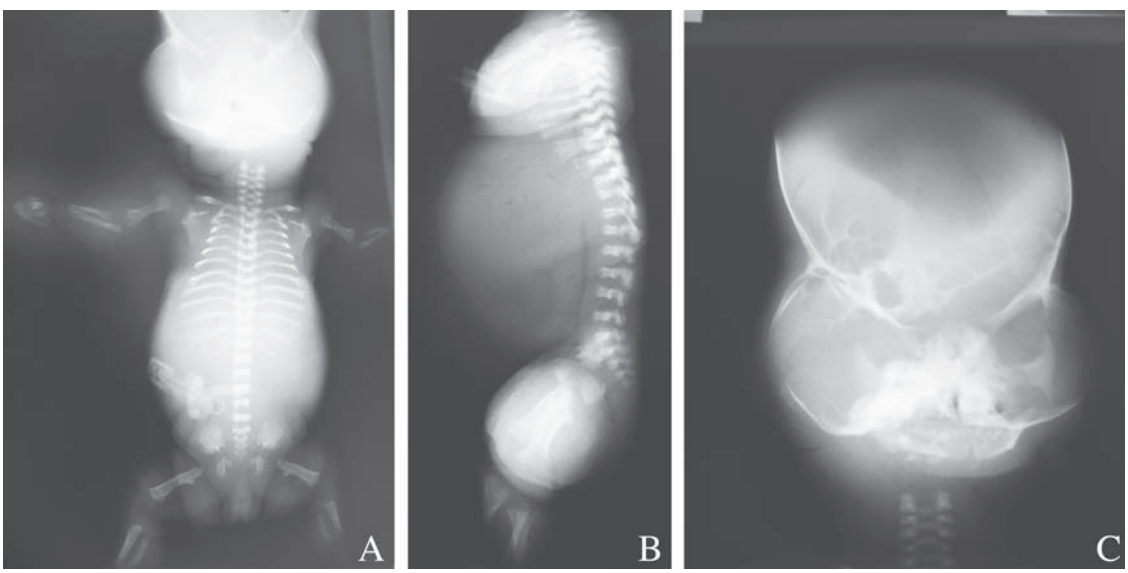


Figure 29.5. Thanatophoric dysplasia type II. (A) Babygram from a 38-week fetus with straight femora, mild platyspondylia, and cloverleaf skull. (B) Lateral view. (C) Detail of cloverleaf skull.

The period of gestation during which a certain type of osteogenesis imperfecta may be demonstrated or excluded by ultrasound varies according to the type of osteogenesis imperfecta. Type IIA, for example, has been diagnosed as early as 15 weeks' gestation (Brons et al. 1988). Although type III is usually nonlethal, it may be very difficult to distinguish from type IIB on the radiographs. In view of this, it has been postulated that type IIB is the severe end of the type III spectrum. Type IIA is the most severe form of osteogenesis imperfecta, and it is characterized on radiographs by severe osteopenia and shortened, severely deformed bones, with a "harmonica" shape, caused by numerous fractures, and contiguous beading of the ribs. The skull is soft because of diminished ossification of the cranial vault (Fig. 29.6). Type IIB and type III show wavy thin ribs and few fractures on the radiographs. The skull bones are thin with scattered small spots of ossification, so-called Wormian bones (Cremin et al. 1982). Type IIC is very rare and characterized by an extreme underossification of the cranial vault, thin ribs with contiguous separate fractures (beaded ribs) and multiple fractures of the long bones. Histological examination of the growth plate cartilage shows no specific abnormalities in the different subtypes, but at the zone of chondro-osseous transformation the primary and secondary (osseous) trabeculae and the cortical bone are reduced in volume with thin osseous seams in types IIA, IIB, and III. There are usually no abnormalities in the cartilage of the growth plate. Sometimes a malformed growth plate can be observed due to fractures close to the growth zone. In the center of the bone very frequently small islands of metaplastic cartilage can be found in all type II cases but are more pronounced in type IIA (Fig. 29.7). Type IIC has a different histological picture. The primary and secondary spongiosum of the metaphysis have a network of relatively broad and irregularly arranged cartilaginous trabeculae with many interconnections and thin osseous seams (van der Harten et al. 1988b).

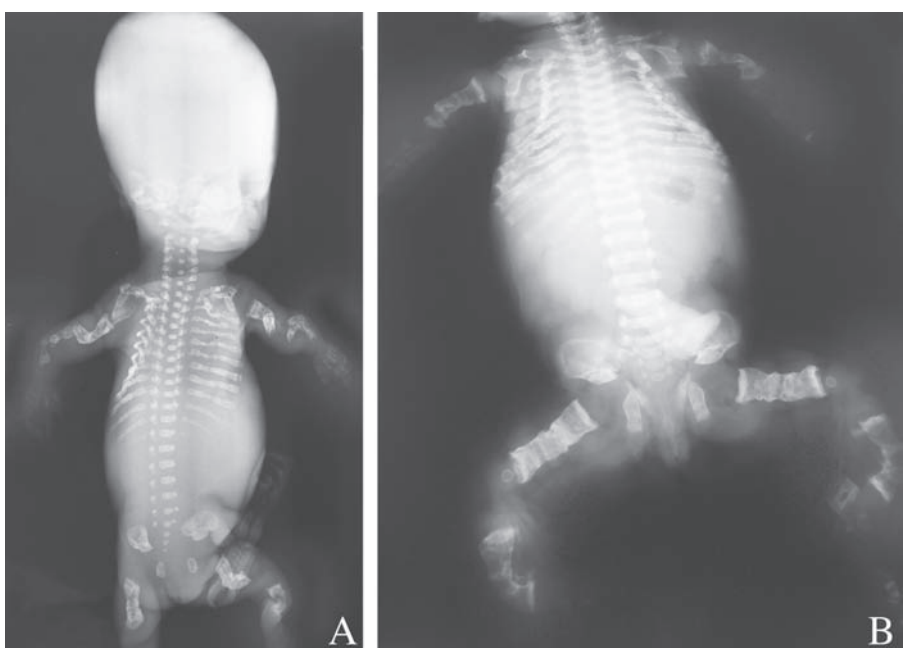


Figure 29.6. Osteogenesis imperfecta IIA. Babygram from a fetus of 22 weeks' (A) and 38 weeks' (B) gestation with severely malformed long bones due to multiple fractures and absent ossification of the cranial vault (A) and multiple rib fractures.

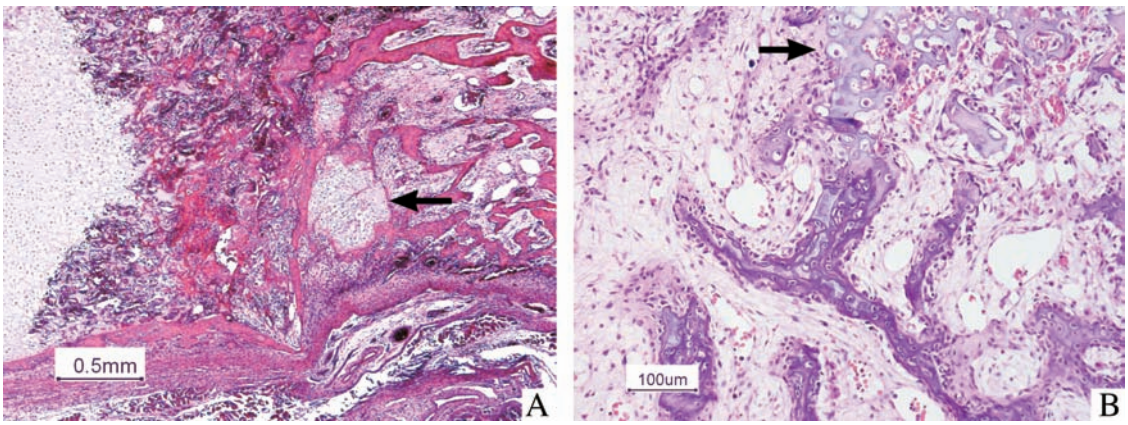


Figure 29.7. Osteogenesis imperfecta IIA. (A) Histology of the epiphysis shows a sharp regular demarcation between cartilage and primary spongiosa. Small islands of metaplastic cartilage (arrow) can be found in the metaphysis. The bony trabeculae are highly cellular and thin and the marrow is focally fibrotic (B).

Recently, another lethal type of osteogenesis imperfecta was discovered, caused by a specific mutation in the carboxyl-terminal propeptide of the pro-α1(I) chain of type I collagen. In contrast to the other types of osteogenesis imperfecta, this type has dense bones. The histology of the epiphyseal growth zone is comparable with the other forms of osteogenesis imperfecta and shows no abnormalities. The primary bony trabeculae, however, are broad and highly cellular, and in the center cartilage matrix can be found, usually without chondrocytes (Pace et al. 2002). The presence of cartilage matrix in bony trabeculae, however, is not specific and can be seen in other types of lethal osteogenesis imperfecta as well, although not as pronounced. In this type of osteogenesis imperfecta there is almost no formation of lamellar bone.

In the differential diagnosis of fractures one must consider the possibility of physical abuse. Analysis of collagen synthesized by dermal fibroblasts may identify children with osteogenesis imperfecta among those suspected of have been abused (Steiner et al. 1996).

In considering the diagnosis of osteogenesis imperfecta, one should always keep in mind the possibility of hypophosphatasia. Several forms of this primary metabolic disorder have been described (Caswell et al. 1989). They are characterized by a reduced activity of alkaline phosphatase in serum and various tissues, including cartilage and bone. The perinatal form has a mortality of 100% (Shohat et al. 1991). As in the lethal type of osteogenesis imperfecta, the skull is soft because of the absence of ossification of the neurocranium. Radiographic examination shows undermineralization of the skeleton and shortening and bowing of the long bones, with angulation and diaphyseal spurs and fractures. In some cases almost no bone is formed (Fig. 29.8). Both the lethal perinatal and the infantile forms have been described within one family (Wladimiroff et al. 1985; Macfarlane et al. 1991).

Histologically, the metaphysis in hypophosphatasia shows irregular bone formation with foci of persisting cartilage islets within the bony trabeculae. Not only chondroid matrix can be found within these trabeculae but also chondrocytes are present. In the diaphysis and close to the metaphysis relatively wide osteoid seams line the bony trabeculae (Fig. 29.9). The picture resembles that of severe rickets (Ornoy et al. 1985b). First-trimester prenatal molecular diagnosis has been reported (Henthorn and Whyte 1995; Orimo et al. 1996).

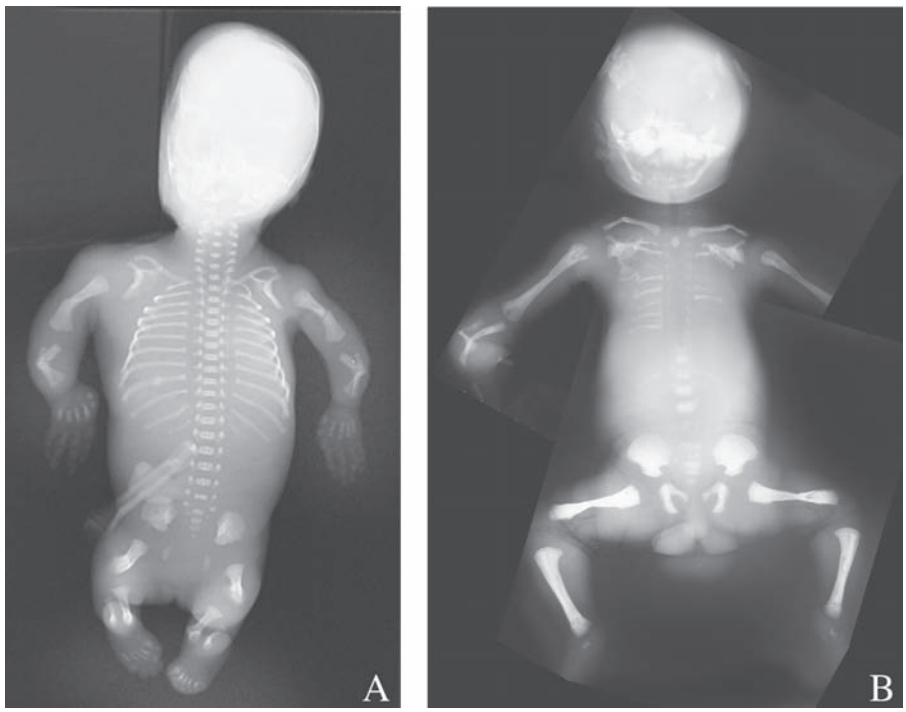


Figure 29.8. Hypophosphatasia. The radiology of hypophosphatasia can be very variable from severe—(A) babygram from a fetus of 38 weeks' gestation—to mild—(B) babygram from a fetus at 23 weeks' gestation. Different bones or parts of bones within the same fetus can be affected to a different extent. Note the irregular distribution of changes in A, with irregular absence of bones or part of bones contrasting with very mild changes in the femur.

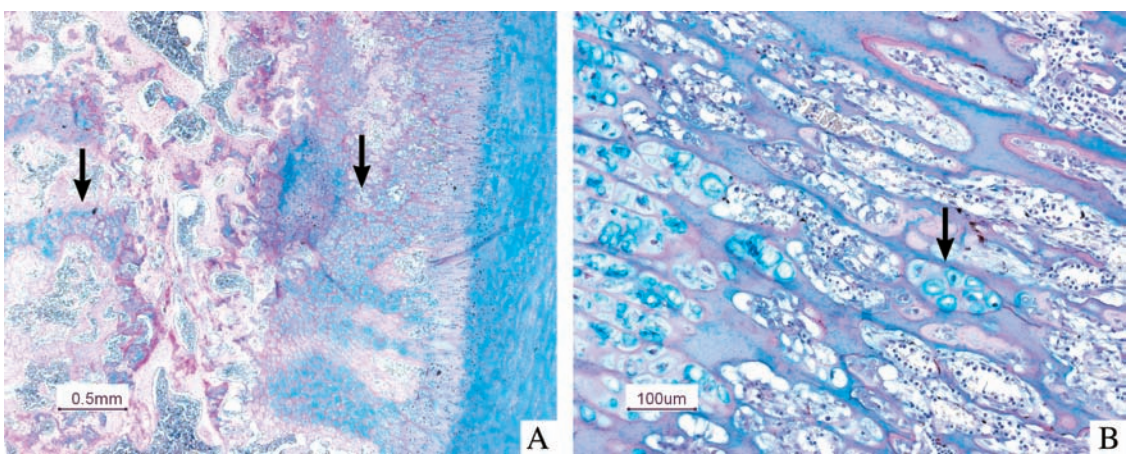
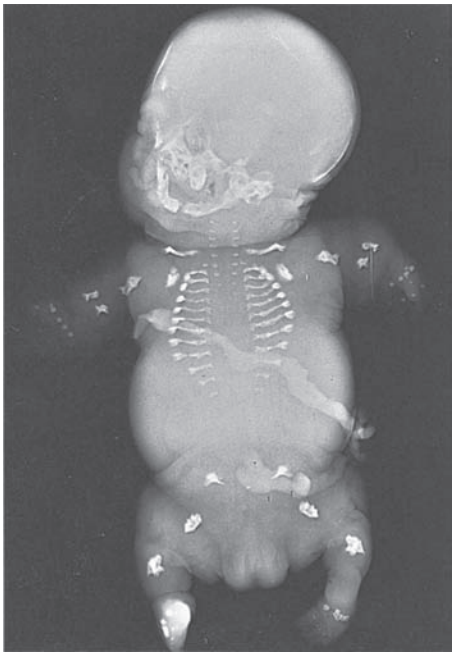
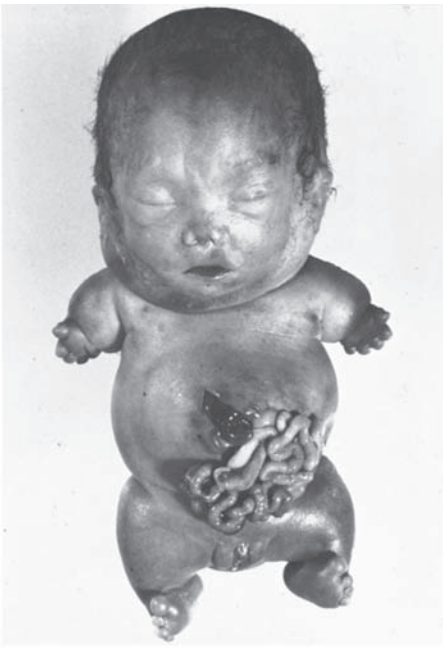
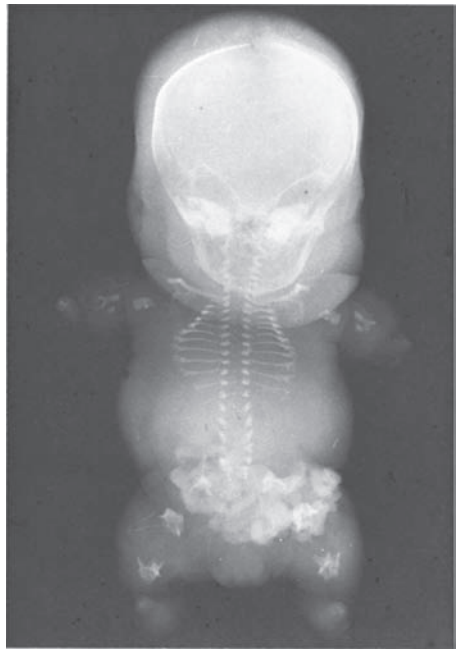


Figure 29.9. Hypophosphatasia. The histology of a vertebra (A) and femur (B) of Figure 29.8A shows extensive irregular bone formation with many large foci of persisting cartilage in bony trabeculae (arrow). The femur shows only a very mild expansion of the cartilage in bony trabeculae, comparable with the less severe abnormalities on x-ray, exemplifying the variability within hypophosphatasia.



A





B

Figure 29.10. (A) Achondrogenesis IA. The babygram of this fetus shows nearly total absence of ossification of the vertebral column, very short malformed long bones with irregular spikes, and thin ribs with some healing fractures. There is absent ossification of the pubic bones. (B) Achondrogenesis IB. The babygram shows absent ossification of the vertebral bodies, short ribs, and very short long bones with metaphyseal cupping. There is absent ossification of the pubic bones.

Type II Collagenopathies

There are several different collagen type II disorders, all with autosomal dominant inheritance. In order of severity, the different types are achondrogenesis type II (Langer-Saldino dysplasia), hypochondrogenesis, and spondyloepiphyseal dysplasia congenita (SEDC). Usually typical cases are observed, but occasionally an overlap between the different types of type II collagenopathies can be seen. In the most recent classification from 2002, Torrance dysplasia was classified within group 1, the achondrodysplasia group, with thanatophoric dysplasia. However, in recent publications a mutation in the carboxy propeptide of the gene for collagen type II was found in cases with Torrance dysplasia (Nishimura et al. 2004; Zankl et al. 2005). This mutation leads to biosyn-

thesis of an altered collagen chain causing a severe phenotype; thus this skeletal dysplasia should be included within the type II collagenopathies with a severity in between that of achondrogenesis II and hypochondrogenesis.

Achondrogenesis Type II (Langer-Saldino Dysplasia)

The phenotype of achondrogenesis II differs little from achondrogenesis type IA and IB, the other two lethal skeletal dysplasias in the radiological differential diagnosis, but development of hands and feet is better (Fraccaro 1952; Langer et al. 1969; Saldino 1971; Borochowitz et al. 1988; van der Harten et al. 1988a). Radiographically, shortening of the tubular bones is not as marked as in both type IA and IB, but disturbance of vertebral ossification is striking (Figs. 29.10 and 29.11). There is absence of ossification of the sacrum and absent or delayed ossification of iliac and pubic bones in achondrogenesis II. The tubular bones are short, with cupping of the metaphysis. There is occasionally coexistent disturbance of organ development apart from pulmonary hypoplasia. Even as in type IA and IB, the skeletal abnormalities of achondrogenesis II are clearly visible by ultrasound in the first and second trimester (Soothill et al. 1993; Meizner and Barnhard 1995). Achondrogenesis IB and not IA, is caused by mutations in the diastrophic dysplasia sulfate transporter gene and will be discussed with the other skeletal dysplasias caused by mutations in this gene (Superti-Furga et al. 1996). The difference of achondrogenesis II from type IA and IB is clear from the histological examination of resting cartilage. The cartilage of achondrogenesis type II is quite cellular and resembles fetal cartilage. Chondrocytes are swollen, and matrix is reduced in volume. Most striking, however, is the 10-fold increase of the vascular channels with abundant perivascular fibrous tissue (Stanescu et al. 1977; Chen et al. 1981; Gruber et al. 1990). The disorganization of the growth plate differs little from that of both types IA and IB, but the formation of columns is focally distinct. Swollen chondrocytes and increase of vascular channels are also found in the cartilages of larynx and trachea. In achondrogenesis type IA the resting cartilage is hypercellular and the chondrocytes

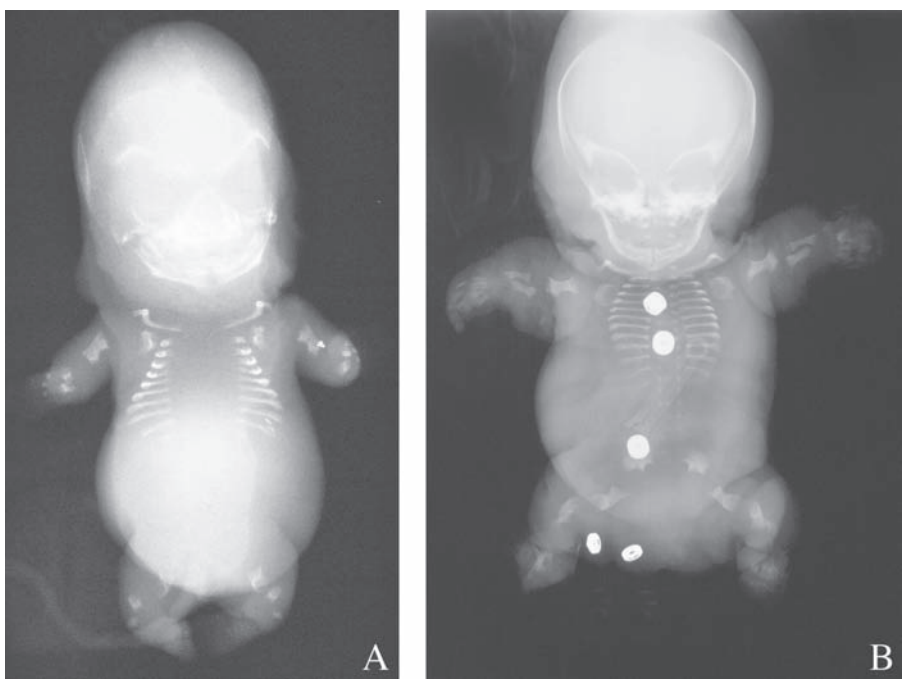


Figure 29.11. Achondrogenesis II. (A) Babygram of a fetus of 14′ weeks gestation. The vertebral column is not ossified, and long bones are extremely short with cupping of the metaphysis. Ossification of the skull is normal. (B) Radiogram of a fetus of 37 weeks' gestation. At this stage, the long bones are extremely short with metaphyseal cupping and absent ossification of vertebral bodies and pubic bones, short ribs, and normal ossification of the cranial vault.

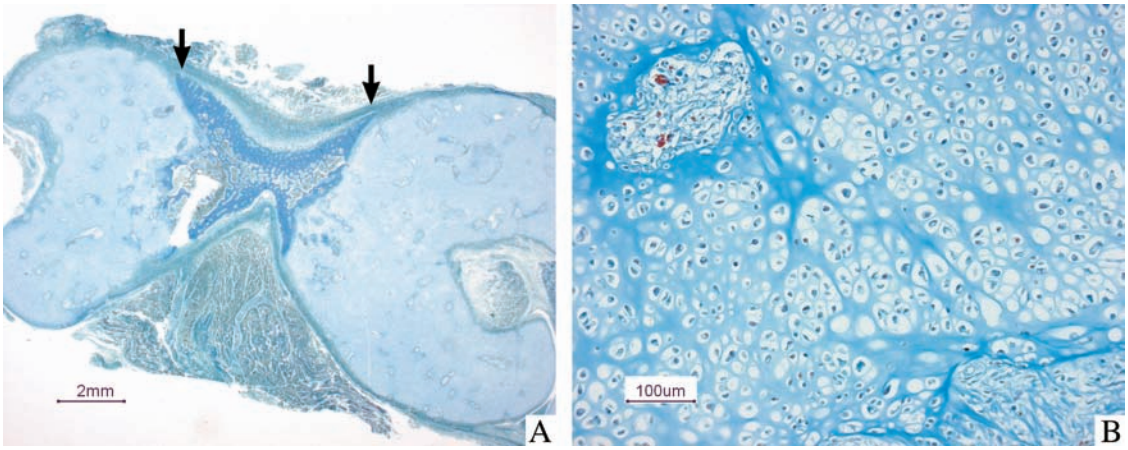


Figure 29.12. Achondrogenesis II. Low- (A) and high- (B) power views of the femur of a fetus of 38 weeks' gestation. Note the huge advanced periosteal ossification that represents the radiological cupping of the long bones (A, arrows). The resting cartilage shows the characteristic but not specific increase in vessels with perivascular fibrosis (B).

contain round or oval periodic acid-Schiff (PAS)-positive, diastase-resistant inclusions (Fig. 29.12). In achondrogenesis type IB intracytoplasmic inclusions are not seen, and the pericellular matrix forms rings around the chondrocytes (Fig. 29.13).

Torrance Dysplasia, Hypochondrogenesis, and Spondyloepiphyseal Dysplasia Congenita

Radiological abnormalities in the Torrance dysplasia are characterized by severe platyspondyly, metaphyseal changes, and brachydactyly. Hypochondrogenesis shows absent ossification of the cervical vertebral bodies and absent ossification of the pubic bones. The long bones are short but not as severe as in achondrogenesis type II, and there is no cupping (Fig. 29.14). Swollen chondrocytes and increase of vascular channels are also found in cartilage of larynx and trachea in hypochondrogenesis, and the epiphyseal growth zone is quite disturbed but not as severe as in achondrogenesis II (Godfrey and Hollister 1988; Feshchenko et al. 1989; Horton et al. 1989; Spranger et al. 1994). These abnormalities can already be easily seen in very young fetuses, for example, at 16 weeks' gestation. In some cases of hypochondrogenesis some chondrocytes have PAS-positive, diastase-resistant inclusions in their

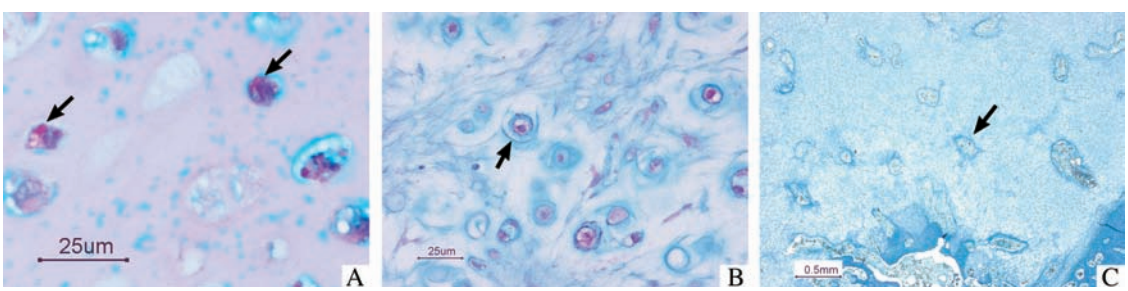


Figure 29.13. Histology of achondrogenesis IA, IB, and II. (A) Typical histology of a case of achondrogenesis IA with round or oval periodic acid-Schiff (PAS) after diastase-positive inclusions in the chondrocyte cytoplasm (arrow). (B) Histology of cartilage in achondrogenesis IB. Rings of collagen surround the chondrocytes in the trichrome stains (arrow). (C) Typical histology of achondrogenesis II with a huge increase of vascular channels, with perivascular fibrosis in resting cartilage (arrow) and disturbed epiphyseal growth zone.

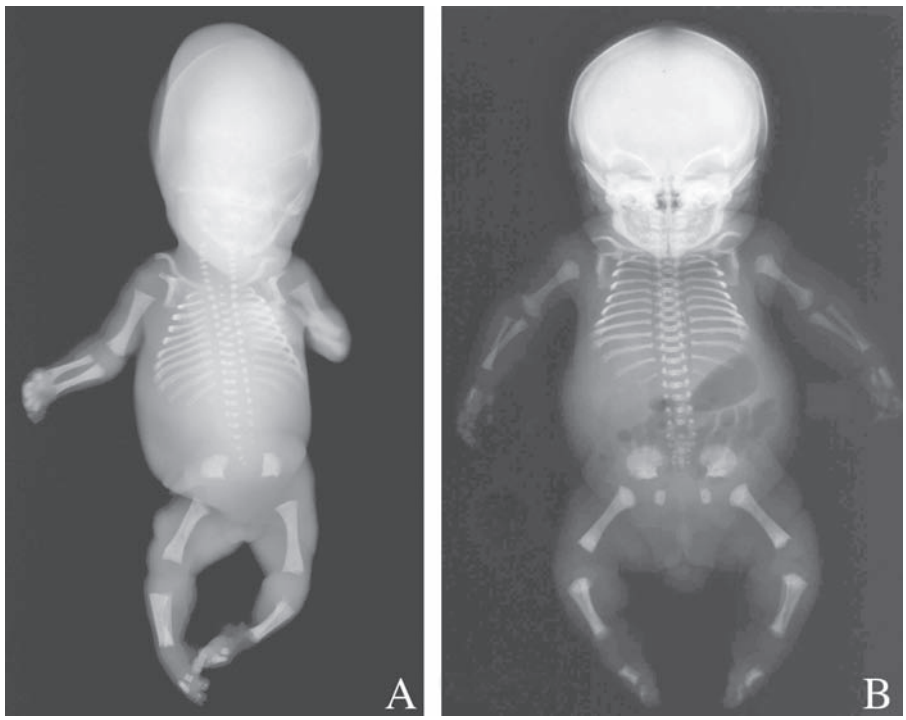


Figure 29.14. Hypochondrogenesis. Babygram of a 19-week (A) and 38-week (B) fetus; short long bones without cupping, absent ossification of the pubic bones, and delayed ossification of the vertebral bodies in the cervical region in the younger fetus (A).

cytoplasm (Fig. 29.15). The chondrocytes in SEDC usually show many PAS-positive after diastase-positive inclusions. Both the increase of vessels in cartilage and the PAS-positive after diastase-positive inclusions are not specific and can be seen in other skeletal dysplasias as well (e.g., Larsen syndrome and Schneckenbecken dysplasia). The histology of the Torrance dysplasia is different. There are no PAS-positive, diastase-resistant inclusions or prominent blood vessels

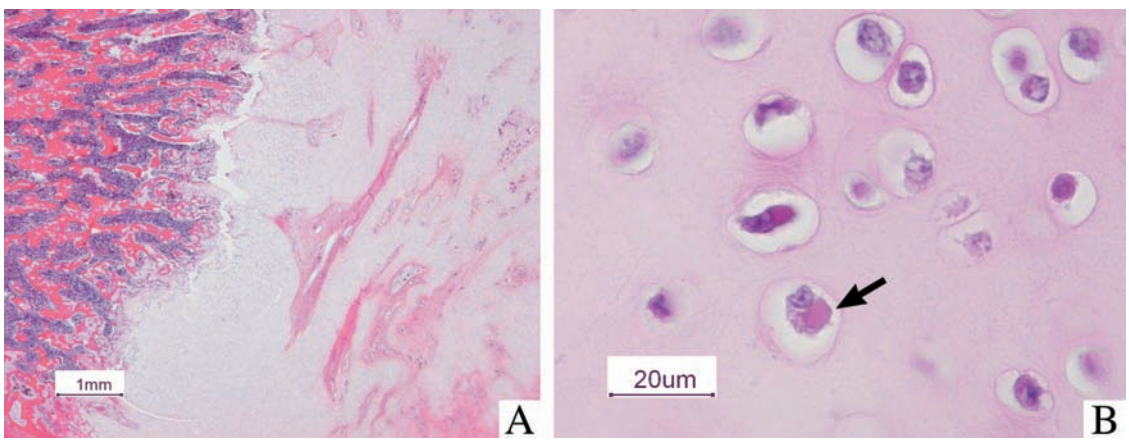


Figure 29.15. Hypochondrogenesis. (A) Histology of the resting cartilage with an increase in vessels with perivascular fibrosis (not as severe as in achondrogenesis II), only moderately irregular epiphyseal growth zone. (B) Occasional PAS after diastase-positive inclusions in the cytoplasm of the chondrocytes (arrow).

with perivascular fibrosis, but the chondrocytes show vacuolization, and the epiphyseal growth zone is slightly irregular (Zankl et al. 2005).

Diastrophic Dysplasia Group

This is a group of skeletal dysplasias caused by mutations in a sulfate transporter. The inheritance is autosomal recessive. The different disorders are achondrogenesis type IB, diastrophic dysplasia, atelosteogenesis type II (cited in the latest classification as de la Chapelle dysplasia), and the nonlethal autosomal recessive form of multiple epiphyseal dysplasia, in decreasing order of severity. The x-rays of achondrogenesis type IB show a severe retarded ossification of the vertebral bodies and absent ossification of the sacrum. The tubular bones are extremely short (see Fig. 29.10). Atelosteogenesis type II has a less severe phenotype with coronal clefts of the vertebral bodies and hitchhiker thumbs. The humeri have a distal V or U shape (Fig. 29.16). Severe lethal cases as well as mild cases have been described for diastrophic dysplasia (Horton et al. 1978). After confirmation of the phenotypic overlap of atelosteogenesis type II with severe diastrophic dysplasia by several investigators (Schrander-Stumpel et al. 1994; Qureshi et al. 1995; Sillence et al. 1997), the genetic relationship was proven with the identification of *DTDST* gene mutations in atelosteogenesis type II in 1996 (Rossi et al. 1996). Diastrophic dysplasia is particularly prevalent in Finland and is characterized by short limbs, spinal deformation, and specific joint abnormalities with hitchhiker thumbs and clubfeet. The histology of this group of skeletal dysplasias is characterized by a variable cyst-like degeneration of resting cartilage, not only of long bones but also in cartilage from other areas like the trachea (Fig. 29.17). It can even be observed in very young fetuses (from 12 weeks gestational age onward). However, this cyst-like degeneration is observed in several other skeletal dysplasias as well, for example, dysseg-

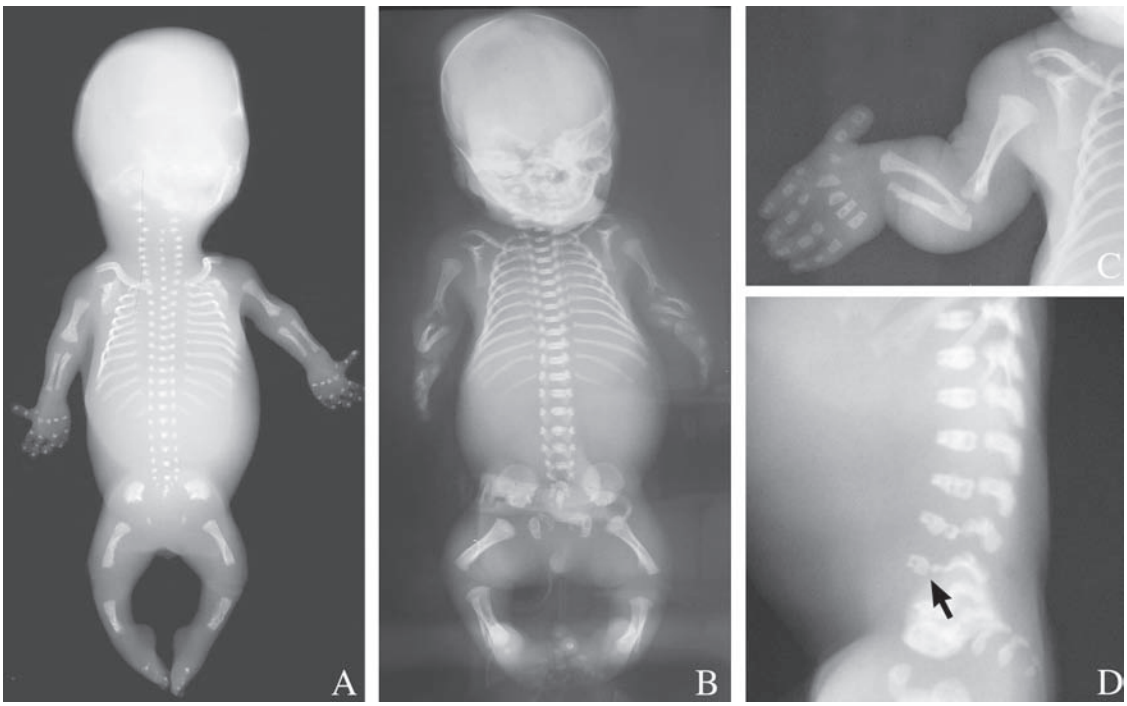


Figure 29.16. Diastrophic dysplasia group. (A) Radiology of a fetus with diastrophic dysplasia at 14 weeks' gestation. (B–D) Radiology of a fetus at 23 weeks' gestation with atelosteogenesis II. The long bones are short and the humerus has a typically V-shaped distal end. The lateral x-ray (D) shows coronal clefts (arrow). Both fetuses show typical hitch-hiker thumbs (A and C).

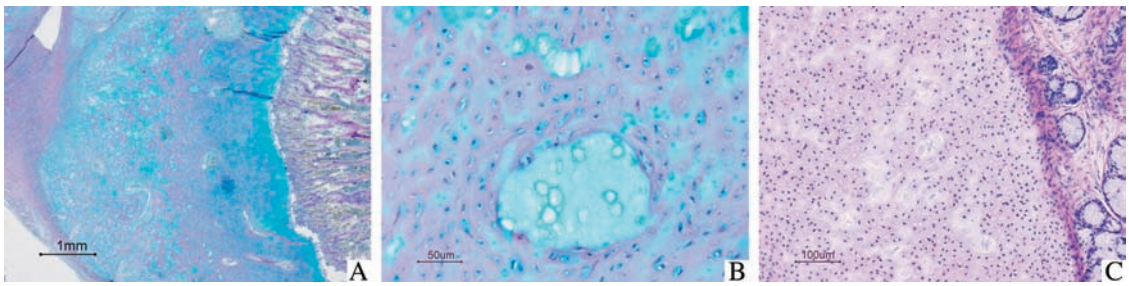


FIGURE 29.17. Diastrophic dysplasia group. Low (A) and high (B) power of the resting cartilage of the femur (PAS Alcian blue stain). (C) Medium power of the laryngeal cartilage (H&E) of a fetus of 23 weeks' gestation. Note the cystic degeneration of the resting cartilage.

mental dysplasia. Rings of dense material surround the chondrocytes in achondrogenesis IB and in the most severe cases of atelosteogenesis II and diastrophic dysplasia. The hypertrophic and proliferation zones appear normal with good columnization.

Short Rib Dysplasias with or Without Polydactyly

Short ribs are present in many different skeletal dysplasias, both lethal and nonlethal types, for example, thanatophoric dysplasia, asphyxiating thoracic dysplasia (Jeune's syndrome), and chondroectodermal dysplasia (Ellis–van Creveld syndrome). The distinction between asphyxiating thoracic dysplasia and the short rib dysplasias is not precise, and in practice depends on the size of the thorax and the length of the infant's survival. These conditions may belong to a spectrum of a pathogenetic entity (Yang et al. 1987). Classification is based on the radiographic appearance (Spranger and Maroteaux 1990; Yang et al. 1980; Saldino and Noonan 1972). These dysplasias are often accompanied by anomalies of other systems. Besides cardiovascular defects, renal cystic dysplasia, ductal plate malformation in the liver, and sexual ambiguity are frequently found. Polydactyly is present in many, but not all, patients. Death is due to pulmonary hypoplasia as a consequence of the extremely narrow thorax. All the cases so far described have exhibited autosomal-recessive inheritance. There is considerable overlap between the different types of short rib dysplasias, but three typical forms of the short rib dysplasias are mentioned in the latest nosology (Hall 2002).

Short Rib-Polydactyly Type I and III (Saldino-Noonan and Verma-Naumoff)

In the condition known as Saldino-Noonan thoracic dysplasia, the narrow thorax, short limbs, and postaxial polydactyly of the hands or feet are striking. Radiographic examination of the skeleton shows extremely short horizontal ribs and a barrel-shaped thorax, dysplastic pelvis, platyspondyly with distorted vertebral bodies, and short tubular bones with very irregular metaphyses (Saldino and Noonan 1972). In about one half to one third of cases, a congenital heart defect, renal cystic dysplasia, and malformed genitals are found; cleft palate is an occasional association. At the growth plate, disorganization of the proliferative and hypertrophic zones with irregular column formation is found on histological examination. The transitional zone from cartilage to bone is irregular, with small groups of chondrocytes localized in the metaphysis and trabeculae of newly formed bone extending into the cartilage. This reflects the irregular radiographic appearance of the metaphysis. Verma et al. (1975) and Naumoff et al. (1977) described several cases with short ribs and polydactyly that they considered distinct from type I on radiographic appearances. Doubt has been expressed about the appropriateness of such separation because of the substantial overlap between types I and III (Yang et al. 1987;

Sillence 1980; Bernstein et al. 1985). The histological appearance of the growth plate parallels metaphyseal irregularity radiographically (Fig. 29.18). Yang et al. (1980) described intracytoplasmic PAS-positive, diastase-resistant inclusions in the chondrocytes of type III, but they do not seem to be universally present (Sillence 1980). In the most recent classification, types I and III have been put together (Hall 2002).

Short Rib-Polydactyly Type II (Majewski)

In addition to the narrow thorax and short limbs, a median cleft lip is usually present. Polydactyly is preaxial and/or postaxial. Radiographically, the ribs are very short, but the spine and pelvis are normal. Unlike type I, the short tubular bones have a smooth metaphysis while the tibia is disproportionately short and oval (Fig. 29.19). Renal cystic dysplasia is found in 50% of cases, but heart defects are rare (Majewski et al. 1971; Chen et al. 1980). Histologically, a regular chondro-osseous transformation zone is seen, which is in keeping with the smooth radiographic appearance of the metaphysis. The proliferating and hypertrophic zones are narrow and have irregular columns.

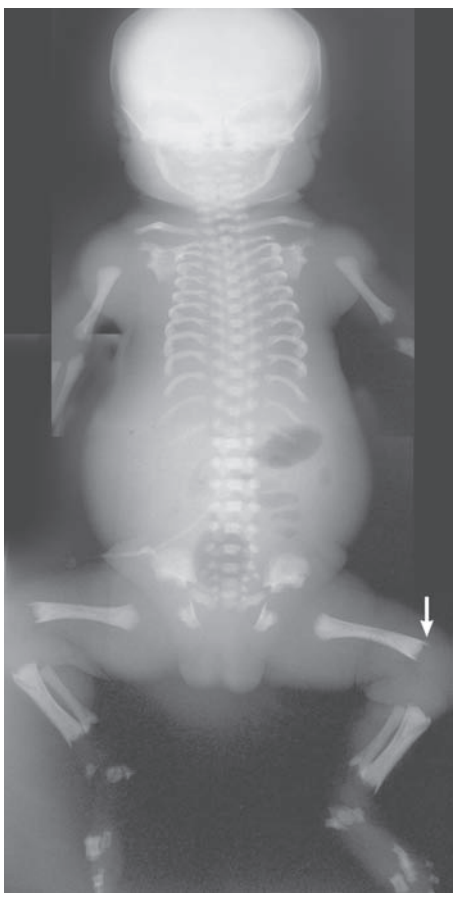


Figure 29.18. Short rib-polydactyly (SRP) type III, Verma-Naumoff. Radiology of a 32-week fetus showing very short ribs, small metaphyseal spikes, and straight long bones.

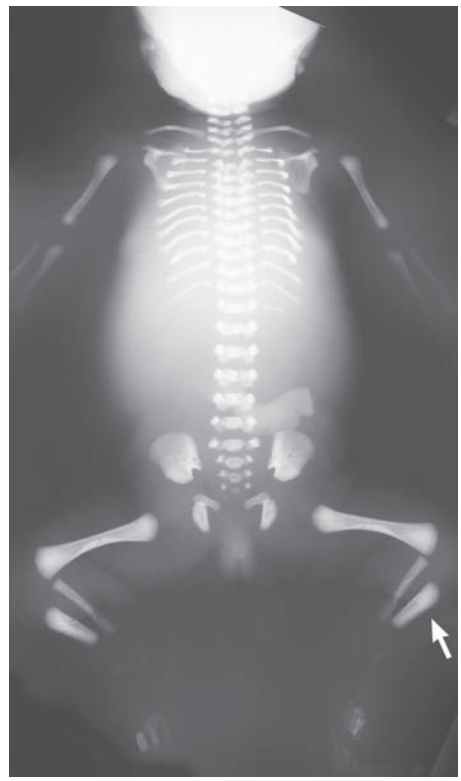


Figure 29.19. SRP type II, Majewski. Radiology of a 32-week fetus demonstrating very short ribs, smooth epiphysis of the long bones, and oval shape of the tibia.

Short Rib-Polydactyly Type IV (Beemer–Langer)

Beemer et al. (1983) reported an additional type of short rib dysplasia showing several characteristics of the other short rib dysplasias, especially of the Majewski type, but without polydactyly. Subsequently it became clear that it represents a distinct entity, and this dysplasia was accepted in

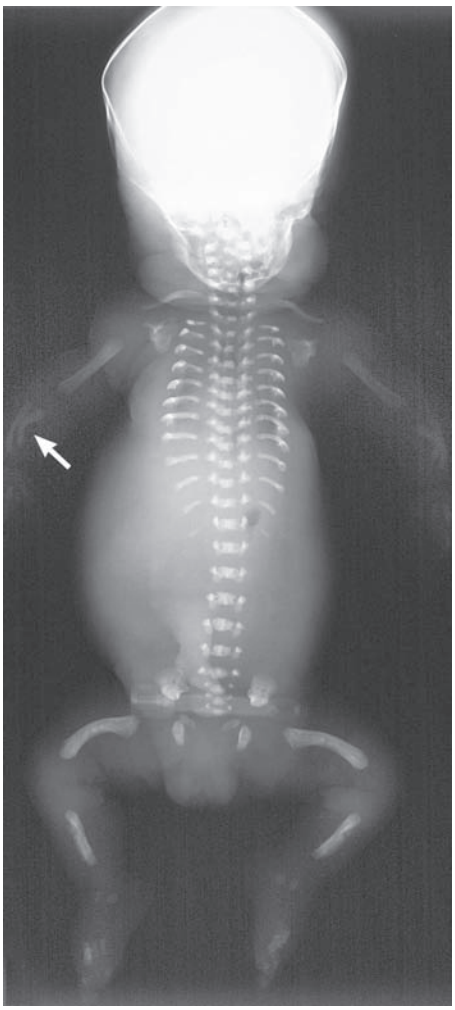


Figure 29.20. SRP type IV, Beemer-Langer. Radiology of a 33-week fetus showing very short ribs, smooth epiphysis of the long bones, and bowing of the femur, radius, and ulna.

the international classification. In contrast with the Majewski type, the Beemer-Langer type shows shorter fibulae than tibiae on the radiographs. The radius and ulna are bowed (Fig. 29.20).

Other Generalized Skeletal Abnormalities Associated with Perinatal Death

Many other skeletal dysplasias are recognizable before or after death; some of them are mentioned briefly as they may be encountered at autopsy. Radiographic examination is important in the investigation of fetuses and babies with these conditions. Many of these dysplasias are inherited in an autosomal recessive fashion, so that all subsequent pregnancies are at risk and ultrasound monitoring is advisable.

Chondrodysplasia punctata, especially the severe, autosomal recessive, rhizomelic type, is a well-recognized disorder in which peroxisomal functions were found to be disturbed (Schutgens et al. 1993). It has to be differentiated from many other conditions in which intra- and extracartilaginous calcifications are present (Table 29.2).

Fibrochondrogenesis is a rare dysplasia, which, in addition to the radiographic findings, can be distinguished with certainty from achondrogenesis by the histological appearance of the resting cartilage. The chondrocytes are spindle shaped, resemble fibroblasts, and are separated into groups by a meshwork of collagen fibers. In the proliferative and hypertrophic zones, formation of irregular columns is seen (Lazzaroni-Fossati 1978; Bankier 1991). The vertebral bodies show a typical configuration on the lateral radiological picture, and the vertebral bodies are pear shaped, with the smaller part pointing dorsally. Occurrence in siblings suggests autosomal recessive inheritance.

Camptomelic dysplasia is characterized by shortening and bowing (angulation) of long bones (femur and tibia), hypoplasia of the scapulae, absent mineralization of the pedicles of the thoracic vertebrae, and a deformed pelvis and spine (Fig. 29.21) (Roth 1991). Affected males with camptomelic dysplasia may have normal genitals, female genitals with streak-like gonadal rudiments, or any stage of male pseudohermaphroditism between these two extremes. Mutations involving the *SOX9* gene have been identified as the basis of this dysplasia. This gene is related to the Y-linked testis-determining factor SrY (Foster et al. 1994; Wagner et al. 1994; Mansour et al. 1995). However, one has to realize that increased curvature of the limbs may occur in many other bone dysplasias (Roth et al. 1982; Houston et al. 1983; Nogami et al. 1986; Roth 1991). Curvature of long bones is also found when there is skeletal involvement in cryptophthalmos (Fraser) syndrome (Thomas et al. 1986). Fraser described a combination of cryptophthalmos and syndactyly

Table 29.2. Lethal skeletal dysplasias grouped according to the International Nosology and Classification of Constitutional Disorders of Bone (2001)

1. Achondroplasia group	
Thanatophoric dysplasia type 1	AD
Thanatophoric dysplasia type 2	AD
Achondroplasia	AD
Hypochondroplasia	AD
SADDAN	AD
2. Severe spondylodysplastic dysplasias	
Lethal platyspondylic skeletal dysplasias (Torrance type, Luton type)	SP
Achondrogenesis type 1A	AR
Opsismodysplasia	AR
3. Metatropic dysplasia group	
Fibrochondrogenesis	AR
Schneckenbecken dysplasia	AR
Metatropic dysplasia (various forms)	AD
4. Short-rib dysplasia (SRP) (with or without polydactyly) group	
SRP type I/III	AR
SRP type II	AR
SRP type IV	AR
Asphyxiating thoracic dysplasia (Jeune)	AR
Chondroectodermal dysplasia (Ellis–van Creveld dysplasia)	AR
5. Atelosteogenesis-omodysplasia group	
Atelosteogenesis type 1 (includes boomerang dysplasia)	SP
Atelosteogenesis type III	AD
6. Diastrophic dysplasia group	
Diastrophic dysplasia	AR
Achondrogenesis IB	AR
Atelosteogenesis type II	AR
7. Dyssegmental dysplasia group	
Dyssegmental dysplasia, Silverman-Handmaker type	AR
8. Type II collagenopathies	
Achondrogenesis type II (Langer-Saldino)	AD
Hypochondrogenesis	AD
Spondyloepiphyseal dysplasia (SED) congenita	AD
Kniest dysplasia	AD
12. Chondrodysplasia punctata (CDP) (stippled epiphyses) group	
Rhizomelic CDP type 1	AR
CDP Conradi-Hünermann type	XLD
CDP X-linked recessive type	XLR
Hydrops-ectopic calcification–moth-eaten appearance (HEM) (Greenberg dysplasia)	AR
Dappled diaphyseal dysplasia	AR
20. Bent-bone dysplasia group	
Camptomelic dysplasia	AR
24. Dysplasias with decreased bone density	
Osteogenesis imperfecta II	AD
Bruck dysplasia I	AR
25. Dysplasias with defective mineralization	
Hypophosphatasia-perinatal lethal and infantile forms	AR
30 Neonatal severe osteosclerotic dysplasias	
Blomstrand dysplasia	AR

Mode of transmission has been noted only if well proven.

Note: After publication of the nosology, Torrance dysplasia ordered in group 2 appeared to be caused by mutations in the collagen type II gene and should be replaced in group 8, the type II collagenopathies.

AD, autosomal dominant; AR, autosomal recessive; SP, sporadic; XLD, X-linked dominant; XLR, X-linked recessive.

Group numbers not contiguous as intervening groups are not lethal.

with additional anomalies including cleft lip and palate; laryngeal atresia or stenosis; and renal, genital, and anal abnormalities. It is inherited as an autosomal recessive condition (Fraser 1962). The frequency of bilateral renal agenesis as part of this syndrome has been underestimated (Lurie et al. 1984).

Homozygous achondroplasia can occur only in the offspring of achondroplastic parents and is almost always lethal in early life.

In metatropic dysplasia there are short extremities, and the long bones show enlarged metaphysis, the so-called barbell-like bones (Beck et al. 1983).

Boomerang dysplasia is a lethal osteochondrodysplasia with deficient ossification of several long bones in the arms or legs, especially the humeri and femora. Some long bones have a boomerang shape (Fig. 29.22). The vertebral bodies are very poorly ossified with coronal clefts on lateral x-ray. Boomerang dysplasia, atelosteogenesis type I, and atelosteogenesis type III represent a spectrum with decreasing severity. The inheritance is autosomal dominant, and the defect is caused by mutations in the cytoskeletal actin binding protein filamin B (Bicknell et al. 2005). The histology of the resting cartilage shows multinucleated large chondrocytes (Fig. 29.20).

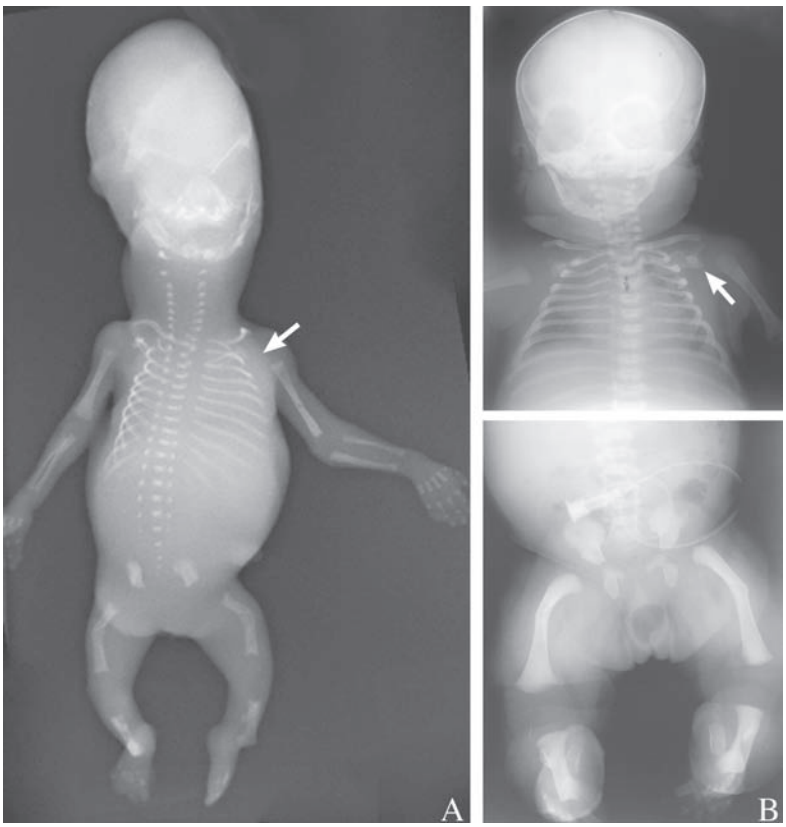


Figure 29.21. Camptomelic dysplasia. Radiology of 16-week (A) and 38-week (B) fetus with typical bowing of the femora combined with hypoplastic scapulae and absent ossification of the pedicles of the thoracic vertebrae.

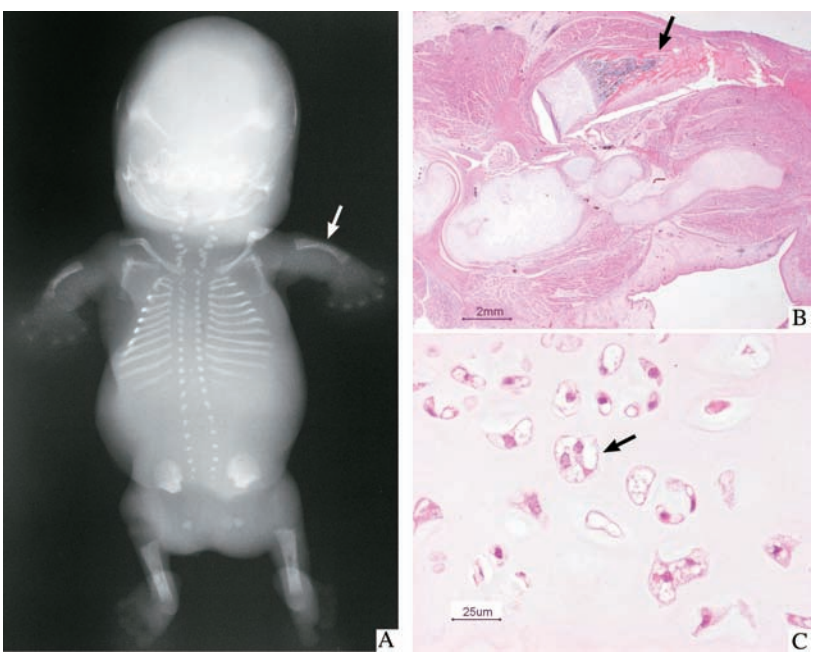


Figure 29.22. Boomerang dysplasia. (A) Radiology and (B) low power microscopy of a 17-week fetus demonstrating absence of several bones of the extremities with bowing of the remaining bone in a "boomerang" shape (usually the tibia and ulna), absent ossification of the vertebral bodies and absent ossification of metacarpal bones. (C) Histology of the same case shows the presence of multinucleated chondrocytes in the resting cartilage, which are also present in the other skeletal dysplasias caused by a filamin B defect.

Fractures

Fractures of skull and long bones are recognized manifestations of birth trauma. Rib fractures may complicate nutritional rickets in preterm babies (Oppenheimer and Snodgrass 1980).

Osteomyelitis

In the neonatal period osteomyelitis is a relatively uncommon complication of a sepsis that is usually caused by *Staphylococcus aureus* or hemolytic streptococci. It has been suggested that *Haemophilus influenzae* type b and *Streptococcus pneumoniae* are also important causes (Faden and Grossi 1991; Jacobs 1991). Outstanding features that distinguish it from osteomyelitis in older children are benign onset and course in most neonates, involvement of multiple foci, and involvement of contiguous joints in cases with long bone infection (Fox and Sprunt 1978; Knudsen and Hoffman 1990). Predisposing factors are complications of pregnancy and delivery. The majority of newborns had antecedent illnesses or were subjected to invasive procedures in the perinatal period (Weissberg et al. 1974). Osteomyelitis of the lower extremity, secondary to catheterization of the umbilical artery, is a well-known complication associated with ischemic changes in the affected limb and infected catheter tip (Lim et al. 1977). Although rare, other modes of infection in neonates include osteomyelitis of the scalp secondary to fetal monitoring and osteochondritis of the calcaneus caused by repeated heel punctures for medical purposes. Osteomyelitis has a predilection for the metaphysis of long bones because bacteria precipitate from the capillaries draining along the cartilaginous growth plate into venous sinuses, rapidly spreading to the subperiosteal space. Early diagnosis is primarily clinical with supporting radiographic findings. When the infection is treated at an early stage, recovery is usually uneventful.

In overwhelming intrauterine infections such as listeriosis, tuberculosis, toxoplasmosis, and candidiasis with intrauterine death, the bone marrow may be involved. The incidence of congenital syphilis, a rare disorder that usually becomes manifest shortly after birth, is increasing (Toohey 1985; Young and Crocker 1994). The pathologist would find more congenital syphilis than current figures indicate, if all fetuses, stillbirths, and placentas were examined histologically (Oppenheimer and Dahms 1981). Characteristic findings include periostitis, metaphysitis, and osteitis involving the long bones, with focal inflammation and fibrosis, the typical syphilitic granulation tissue. Adequate and early treatment provides prompt reversal of symptoms and radiographic findings (Ikeda and Jenson 1990).

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30
The Skin

Peter R. Millard and Fraser G. Charlton

Development of the Skin

The development of the epidermis is outlined in Table 30.1.

Periderm

The periderm presents as large a surface area as possible to the amniotic fluid (Fig. 30.1). This large area and the pinocytic vesicles suggest a secretory or absorptive function, and it is interesting that the amniotic fluid composition changes coincidentally with the loss of the periderm and onset of keratinization (Holbrook and Odland 1975). Prior to keratinization, the peroderm's composition resembles that of the fetal extracellular space. With keratinization, the permeability of the fetal skin is reduced and the amniotic fluid resembles fetal urine, with an increased concentration of urea and creatinine and a decrease in sodium and glucose (McCarthy and Saunders 1978).

Keratinocyte

Keratinocytes constitute the main cell population of the epidermis arising from cuboidal basal cells and flattening progressively as they migrate toward the skin surface. These cells are bound together by desmosomes, seen as prickles on light microscopy, formed at the cell membrane by dense plaques and converging intracytoplasmic tonofibrils.

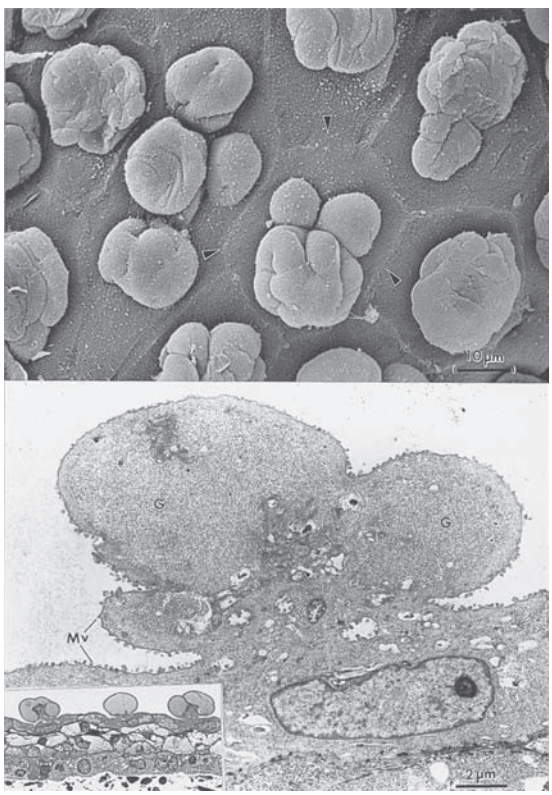
Tonofilaments, often mistakenly regarded as keratin fibrils, are synthesized by the epidermal cells together with sulfur-rich keratohyaline granules. Both increase in size as these cells become more superficial and the keratohyaline granules are aligned between and around the tonofilaments. The tonofibrils form the cytoskeleton of the keratinocytes and are pliable. The keratohyaline granules provide rigidity to these cells by contributing to the amorphous firm matrix of the cells, particularly those of the granular layer where keratinization is complete (Elias 1989).

Molecular analysis of tonofibrils has revealed a number of subtypes that are broadly divided into type I and type II keratin molecules, each produced by a single gene. The types differ in their molecular structure in that type I are low weight (40 to 64 kd) and type II high weight (52 to 68 kd). They also differ in their distribution in both normal and abnormal cells, where more than one example of each subtype can occur. Absence of expression has been correlated with some disorders, notably forms of epidermolysis bullosa. Their presence can be revealed by immunostaining with labeled cytokeratin antibodies. The intercellular keratinosomes, or Odland bodies, should not be confused with the intracellular keratohyaline granules. Odland bodies are lamellated masses that develop from the cell membranes of the granular and more particularly the horny layer cells and lie in the intercellular spaces. These regions are filled with lipids, which are derived from the keratinosomes and both limit water loss and promote cell cohesion.

Table 30.1. Development of the skin

Conceptual age	Crown–rump length (mm)	Description	Ultrastructure[a]
<5 weeks	<10	Single layer of cells—ectoderm	Few small microvilli on free surface. No desmosomes
5–8 weeks	10–30	Bilaminar, upper periderm, lower germinative. Occasional non-keratinocyte.	Microvilli and pinocytic vesicles on periderm surface. Immature desmosomes; no hemidesmosomes. Basal lamina present. Peripheral tonofiliments
8–11 weeks	30–65	Incomplete layer of interstitial cells. More melanocytes	Periderm large cells with central elevation. Many surface microvilli with surface fuzz of mucopolysaccharide
9–13 weeks	40–95	Trilaminar epithelium. Early hair germs	Periderm cells have large surface bulb covered by microvilli
12–16 weeks	80–140	Two or more layers of interstitial cells	Periderm cells stop dividing and expand laterally. Surface still has a bulb, microvilli and pinocytic vesicles
13–17 weeks	95–150	As above	Periderm shows complex surface infoldings and protuberances. Interstitial cells accumulate tonofilaments
16–23 weeks	140–185	Four or more layers of interstitial cells. Keratinisation first seen around hair follicles	Many periderm cells lose nucleus and internal structure. Periderm begins to peel away. Upper interstitial cells contain keratohyaline
>23 weeks	>185	Appearance approaches adult epidermis	

[a]Hashimoto et al. (1966), Wessells (1967), Hoyes (1968), Breathnach and Robins (1969), Breathnach (1971), Holbrook and Odland (1975).



Melanocytes

Neural crest cells are recognizable at 18 days post-fertilization and seen as aggregates along the neural tube at 25 days. They migrate to the periphery and are found in the dermis around vessels and nerves. Melanosomes are recognizable in epidermal melanocytes at 8 weeks and melanin pigment at 10 weeks. Migration and maturation appears to occur slightly earlier in cranial than in caudal regions (Sagebiel and Odland 1970).

Similar numbers of peripheral melanocytes are present in pigmented races and Caucasians, but in the former they are more active (McDonald

Figure 30.1. Periderm of a 14-week fetus. Top: Scanning electron microscopy of the sole of the foot. Individual periderm cells with complex surface protuberances are evident, with arrowheads indicating the cell margins. Bottom: Transmission electron microscopy of the same area with thin section light microscopy. Inset: Microvilli (MV) extend from the surface of the protuberance and glycogen (G) is abundant in the cytosol. The trilaminar epithelium supporting the protuberances is seen. (Courtesy of Dr. D. Ferguson, Oxford, England.)

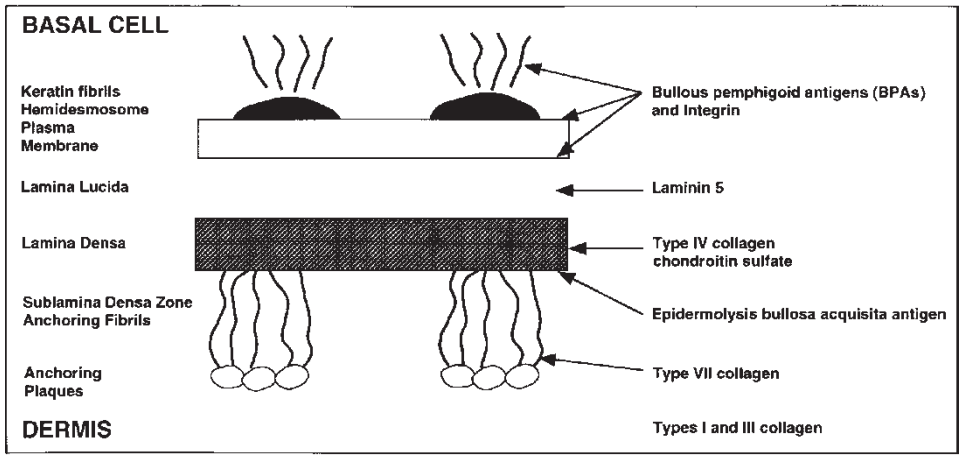


FIGURE 30.2. Components of the dermal–epidermal junction and the location of the main proteins. Using antibodies to these proteins the site of some skin disorders can be localized (Eady 1987; Rasmussen et al. 1989).

1988). These differences do not affect the spectrum of cutaneous disorders among black neonates (McLaurin 1988; Nanda et al. 1989). S100 antibody will demonstrate melanocytes in tissues and HMB45 antibody will demonstrate fetal melanocytes, active melanocytes in melanocytic nevi as well as those constituting melanomas. MelanA is a more recently developed antibody with a high specificity for melanocytes that is generally more useful than HMB45 in the diagnosis of melanocytic lesions (Jungbluth et al. 1998).

Langerhans' Cells

Langerhans' cells are cells of the monocyte/macrophage series. They are potent antigen-presenting cells, able to stimulate T cells, particularly in response to surface antigens on keratinocytes and melanocytes (Shimada and Katz 1988; Stingl and Steiner 1989; Bos and Kapsenberg 1993). They form 2% to 8% of the epidermal cells and occur in smaller numbers in the dermis. The cells are identified by characteristic rod- or tennis racquet–shaped Birbeck granules and have been seen in the epidermis of a 12-week postfertilization embryo (Breathnach and Wylie 1965). The granules are lacking in otherwise similar forms—indeterminate cells. All cell forms are OKT6 and CD4 positive and express class II antigens.

Dermis and Dermoepidermal Junction

The dermis develops from loosely arranged mesenchymal cells at 5 weeks to a pattern mimicking the adult form, including appendages, by the end of the first trimester (Smith and Holbrook 1986). A similar pace of development occurs at the dermoepidermal junction, where the many basement membrane zone proteins are formed (Smith et al. 1988) (Fig. 30.2) and binding between the basal cells and basement membrane occurs (Yancey 1995).

Skin Function

The functions of the skin of the neonate are identical to those of the adult but are modified in several important ways. Microbial colonization begins at birth, and is equivalent to that of the adult only after several weeks of life. The protective antagonism of the resident flora to microbial pathogens is consequently absent, and different patterns of skin infection can be anticipated (Roth and James 1989). Exposure to other antigens has not occurred, and immune responses to environmental antigens are consequently not developed. A minority of infants, however, have been exposed to harmful antigens in utero and may as a result manifest skin infections, neoplasms, and

immunologically mediated disorders at birth (Kruisinski and Saurat 1989).

Heat loss is greater in the neonate because of the large surface area of the skin relative to volume, the small amount of subcutaneous fat, and immaturity of vascular tone regulation. Conversely, the poor responses of the eccrine glands preserve heat and even overheat the infant (Atherton and Rook 1986).

Disorders of Development

Amniotic Constriction Bands

If the amnion ruptures in the first half of pregnancy without rupture of the chorion, the exposed extraembryonic mesoderm can adhere to the embryo. Fibrous strings are sometimes formed, which may encircle parts of the fetus and give rise to constriction bands. When circulation is seriously impaired, amputation of parts of limbs, syndactyly, cleft lip, anencephaly, and encephaloceles may result (see Chapter 6). The reported incidence is 1 in 10,000 live births, and 1 in 5000 total births and abortions. Amniocentesis may also result in amniotic constriction bands (Gellis 1977).

Small skin pits are occasionally encountered in the neonate, and some may arise from direct trauma during amniocentesis (Fig. 30.3).

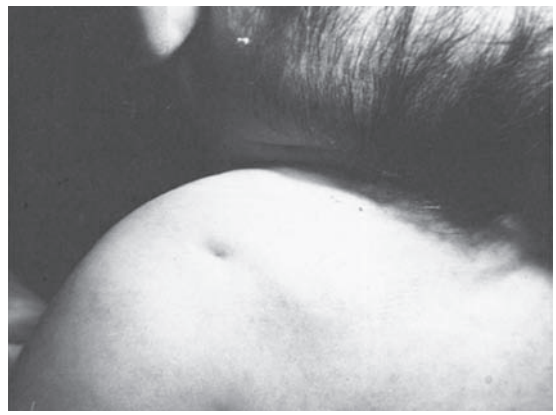


Figure 30.3. Congenital dermal pit on the shoulder. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

Dermoid Cysts

Dermoid cysts are congenital cystic structures with a wall of keratinizing epithelium possessing adnexae, and are thought to be a developmental abnormality occurring at sites of closure of developmental planes. Of congenital dermoid cysts, 37% are in an orbital or periorbital site and 80% are on the face and neck (Pollard et al. 1976). The cysts may be large and may occasionally extend through the bone of the orbit or face.

Accessory Auricle

Accessory auricles/tragi are dome-shaped papules, usually solitary, found predominantly in the preauricular region, but may also be present on the neck. Histologically, they comprise epidermis and dermis, including hair follicles, overlying a fibroadipose core. Classically, there is a bar of mature cartilage within the fat, but this is not always present (Satoh et al. 1990).

Congenital Sinus

Epithelial-lined sinus tracts are most commonly seen in the midline in the neck and the lumbosacral area tethered to the filum terminale. A hairy tail is occasionally present in the lumbosacral area (Fig. 30.4).

Aplasia Cutis Congenita

This is a very rare disorder with multiple subtypes and associated patterns of inheritance. The primary feature is a localized absence of epidermis, dermis, and subcutis, most usually on the scalp (Fig. 30.5). Healing of small lesions takes place with scarring and focal alopecia. The presence or absence of other features determines the subtype, and can include limb abnormalities, epidermal or organoid naevi, and embryological malformations (Frieden 1986). It is most commonly encountered by the pathologist in the fetus or baby with trisomy 13.

Ectodermal Dysplasia

Ectodermal dysplasia is a heterogeneous group of congenital developmental abnormalities of structures derived from ectoderm, principally hair, nails, teeth, and sweat glands, that are generalized

Figure 30.4. Congenital hairy tail; these may be seen overlying spina bifida occulta.

and nonprogressive (Solomon and Keuer 1980). The best defined is anhidrotic (also called hypohidrotic) ectodermal dysplasia (AED), a recessive X-linked disorder associated with the *LEDA* gene, localized to Xq11–12 (Kere et al. 1996), in which there is failure to sweat; complete or partial anodontia; thin, sparse growth of body and scalp hair; and a characteristic facies, with prominent forehead, brow ridges, and a pointed chin. Sometimes there is nail dystrophy, hyperkeratosis of palms and soles, cleft lip, and absence of the mammary glands. Full expression occurs only in males, and female carriers show mild, patchy epidermal changes (Martin-Pascual et al. 1977). Histologically, there is a marked reduction in the number and size of eccrine glands, hair follicles, and sebaceous glands (Martin-Pascual et al. 1977; Arnold et al. 1984). The disease can be diagnosed in male fetuses by prenatal skin biopsies (Arnold et al. 1984). Several biopsies must be taken, and, although the normal development of sweat glands is not complete until 22 to 24 weeks, lack of normally developing hair follicles and sebaceous glands is diagnostic.

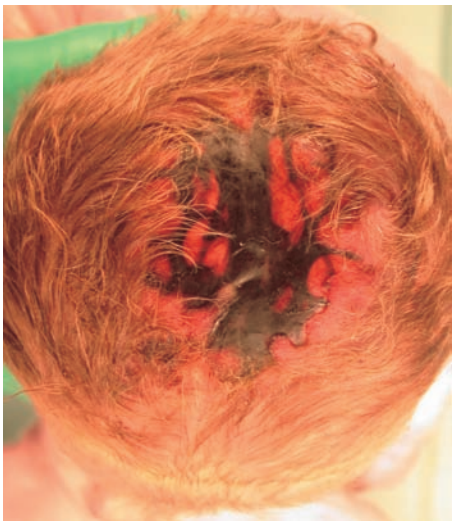


Figure 30.5. Aplasia cutis congenital in the scalp in trisomy 13; blood vessels and cerebral gyrae are seen though the thin membrane.

Another well-documented disorder is hidrotic ectodermal dysplasia (Clouston type) with autosomal-dominant inheritance, characterized by sparse, thin hair; hyperkeratosis of palms and soles; nail dystrophy with or without dental abnormalities; pigment disturbance; and central nervous system (CNS) involvement. There is a marked reduction in the numbers of hair follicles, with abnormalities of existing hair bulbs and hyperkeratosis and acanthosis of the palms and soles (Pierard et al. 1979). Mutations in the gene *GJB6* have been implicated in the development of this condition (Lamartine et al. 2000)

Rare ectodermal dysplasias are reviewed by Solomon and Keuer (1980) and Pinheiro and Freire-Maia (1983), and a new classification is proposed by Lamartine (2003).

Dermal Developmental Disorders

Ehlers-Danlos Syndrome

Several inherited conditions referred to collectively as the Ehlers-Danlos syndrome show varying degrees of skin hyperextensibility, fragility, hypermobility of joints, and aneurysm formation. They are all associated with abnormalities of collagen. The skin bruises easily and may be thinner than normal, often by as much as half, and collagen depleted. The collagen fibrils may be larger or smaller and mixtures are common (Sulica et al. 1979). Elastic fibers, because of the loss of collagen, can falsely appear increased. Electron microscopy reveals alterations in collagen fiber diameters (Black et al. 1980; Holbrook and Byers 1982).

Collagen provides the mechanical scaffolding for many tissues and at least 11 types are described. The interstitial collagens are types I to III, of which type I is the most abundant and the major type in the adult dermis. Type III collagen is usually associated with type I and comprises 50% of the collagen of fetal dermis. After birth, less than 20% of the dermal collagen is type III. Types IV and VII are constituents of the basement membrane zones. The Ehlers-Danlos disorders are related to changes in the types I and III collagens or associated enzyme deficiencies (Mao and Bristow 2001).

At least 10 main variants of the disorder exist. Type IV Ehlers-Danlos syndrome, a severe disease with a high incidence of fatal rupture of large arteries, is due to a variable deficiency of type III collagen. This deficiency is associated with mutation of the type III collagen gene and may be recognized antenatally (Pope et al. 1988). It is type IV Ehlers-Danlos that can present in the neonatal period with skin, ocular, and skeletal deformities. These are due to a deficiency of lysyl-hydroxylase, which converts lysine residues within the molecule to hydroxylysine, important for stable covalent cross-links between collagen molecules during fiber formation.

Cutis Laxa

The term *cutis laxa* covers a spectrum of rare conditions, all of which entail loose and inelastic skin. The inheritance may be autosomal recessive (most commonly), dominant, or sex linked. Abnormally loose skin is sometimes apparent at birth, and the infant may develop systemic involvement, including emphysema, which may cause death in infancy (Ledoux-Corbusier 1983). These babies may have lysyl-oxidase deficiency and large collagen fibrils (Pope and Nicholls 1986). There is a primary defect in elastin fibers, with a variety of mutations reported (Khakoo et al. 1997; Moller et al. 2000; Rodriguez-Revenga et al. 2004). Histologically, dermal elastic fibers are diminished and may appear tapered or fragmented, although they can appear normal, particularly in the early stages.

Focal Dermal Hypoplasia (Goltz's Syndrome)

Focal dermal hypoplasia is rare, almost always found in females, and characterized by multiple focal lesions in which the dermis is extremely thin. Many children have systemic abnormalities, especially skeletal (80%) and ocular (40%). The skin lesions are apparent at birth as atrophic red scars and become worse with age. In places, subcutaneous fat protrudes through the deficient dermis. Histologically, the dermis is focally thin and the collagen fibers have smaller diameters but show normal striation. Connective tissue is reduced but fat cell content is maintained (Lawler and Holmes 1989). The female preponderance raises the possibility of a dominant gene on the X chromosome that is usually lethal in males (Goltz et al. 1970; Gottlieb et al. 1973).

Developmental Abnormalities of Pigment

Albinism and Piebaldism

Oculocutaneous albinism is characterized by variable loss of skin pigmentation, translucent irides, and hypopigmentation of the retina. The term encompasses a heterogeneous group. The incidence is 1 in 200,000 in the United States, but is much more frequent in some societies. There are normal numbers of melanocytes with melanosomes, but reduced or absent melanin production. The most severe subtype is due to mutations to the tyrosinase gene, which can be detected prenatally (Shimizu et al. 1994). There is an increased incidence of malignant skin tumors.

Piebaldism, or partial albinism, has autosomal-dominant inheritance and shows well-circumscribed areas of depigmentation that often have a characteristic and almost symmetrical pattern. A lock of white hair on the forehead is commonly seen in this disorder. Melanocytes are absent in the epidermis and dermis of the affected areas, and this may be due to a defect in the migration of melanocytes into hair follicles (Tomita 1994).

Common Neonatal Skin Conditions

Most of these conditions can be diagnosed clinically. Biopsy is rarely resorted to unless complications, principally infection, have developed. In

these circumstances a small punch biopsy with multiple levels is often appropriate.

Erythema Toxicum Neonatorum

This is a diffuse rash of unknown cause that never affects the palms and soles and occurs in 30% of mature babies. It is usually seen at age 2 to 3 days and is self-limiting, with a blotchy erythema and superimposed pustules. The baby is well but a dermal infiltrate of eosinophils is seen, especially around hair follicles, with papillary dermal edema; the pustule is a subcorneal bulla filled with eosinophils and close to one or more hair follicles. There is sometimes a peripheral blood eosinophilia (Luders 1960). A small proportion of neonates, fewer than 5% of black infants and even fewer white infants, progress from a transient disorder, transient pustular melanosis, to erythema toxicum neonatorum. The histology of the two conditions is similar, but after the pustules of pustular melanosis rupture, hyperpigmented macules remain for some time. A graft versus host reaction from maternal lymphocytes has been suggested as the cause of erythema toxicum (Bassukas 1992), but histologically the changes are not typical.

Nappy Rash

Nappy rash accounts for 20% of skin consultations in infants and young children (Honig 1983). The "primary irritant" type is caused by a tight-fitting nappy and plastic pants leading to moist skin, maceration, and friction damage, exacerbated by the irritant effect of ammonia and proteolytic fecal enzymes. There are red, shiny, glazed areas sparing the inguinal folds (Fig. 30.6). Nappy rash caused or prolonged by *Candida albicans* consists of clusters of erythematous papules and pustules coalescing into a red rash (Fig. 30.7). *Candida* can be cultured from the skin in 40% of babies with nappy rash, and rarely this is complicated by generalized plaques all over the body—the Id reaction. This reaction is thought to have an immunological basis, as *Candida* cannot be cultured from lesions other than in the nappy area. Nappy rash is more common in bottle-fed babies and in babies with a family history of atopic dermatitis or seborrheic eczema. The differential diagnosis includes histiocytosis X, infantile psoriasis (Farber et al. 1986), scabies, and allergic contact dermatitis. Granuloma gluteale can be a complication (Walsh and Robson 1988).

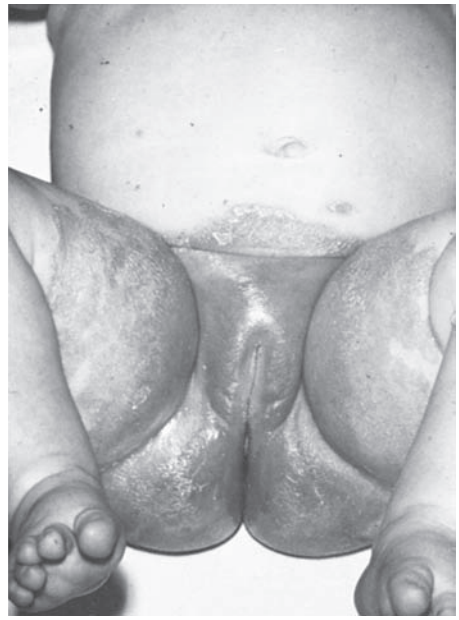


Figure 30.6. Napkin dermatitis. There is scaling and red, shiny glazed areas. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

Milia

These are tiny white papules seen on the forehead and face of up to 50% of infants (Fig. 30.8). They

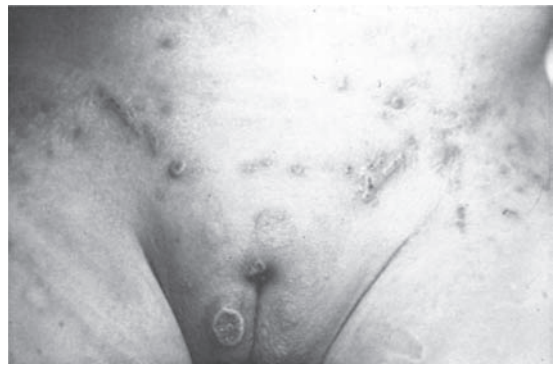


Figure 30.7. Napkin dermatitis with raised circumscribed pustules and papules often associated with *Candida albicans* infection. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

disappear during the first 4 weeks of life and are due to retention of keratin and sebaceous material within the pilosebaceous apparatus or eccrine sweat ducts.

Neonatal Acne

This acne-like eruption develops within a few days of birth in 20% of neonates, almost always boys, and is confined to the face (Fig. 30.9). The spots disappear in the great majority of babies. The etiology is unclear, but stimulation of facial pilosebaceous units by fetal androgens may be responsible.

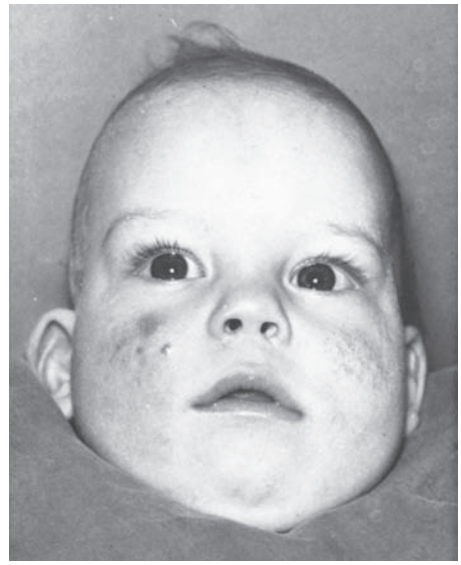


Figure 30.9. Neonatal acne. Males are usually affected. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

Miliaria

Miliaria is very frequent in the neonate, particularly in a hot, humid environment, for example, incubators. It is a symmetrical micropapular and vesicular rash occurring on the face, neck, and trunk. Clinically, it takes two forms: sudamina and prickly heat. Recurrent crops develop and resolve after 2 to 3 days. Sweat retention from the relative immaturity of the eccrine glands and ducts is the cause. The result is edema around the ducts in the epidermis, sometimes with vesicle formation, and a mild mononuclear cell infiltrate.

Infantile Seborrheic Dermatitis

An erythematous scaly rash beginning on the scalp (cradle cap) in a neonate suggests infantile seborrheic dermatitis and is due, in part, to increased sebaceous gland activity (Fig. 30.10). The features are those of a subacute spongiotic dermatitis, sometimes with parakeratosis containing neutrophil debris that resembles psoriasis. No relationship with the adult disorder is known.

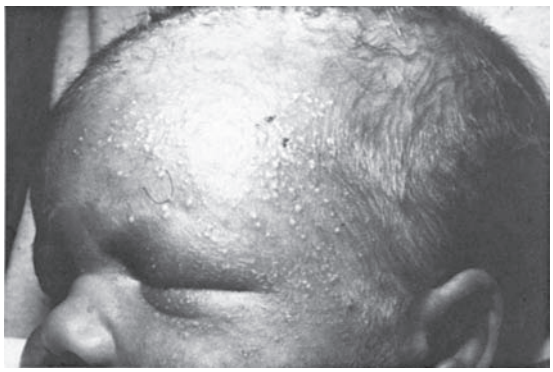


Figure 30.8. Milia. Clusters of small white papules characteristically seen on the upper part of the face. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

Subcutaneous Fat Necrosis

This self-limiting disorder, usually resolving within 2 to 4 weeks of birth, is seen in full-term, often postmature, babies. Single or multiple circumscribed indurated small nodules to large plaques, rarely painful, are evident on pressure areas. Within the involved fat is a lobular panniculitis with necrosis of fat cells, foreign body–type giant cells, a lymphohistiocytic infiltrate, and needle-shaped crystals within the fat and histiocytic cells (Friedman and Winkelmann 1989). Rarely, calcification and liquefaction supervene, in which case scarring will follow. Trauma, asphyxia, hypothermia, and maternal diabetes have all been advanced as causative, although these conditions may only serve to unmask a basic defect in the composition and metabolism of the fat (Friedman and Winkelmann 1989). The condition may also be associated with hypercalcemia,

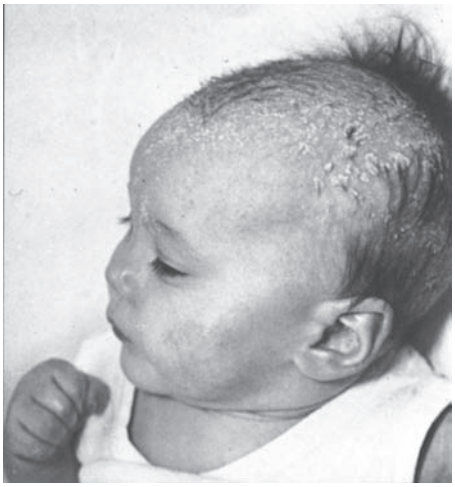


Figure 30.10. Infantile seborrheic keratosis. There is extensive crusting of the scalp and some loss of hair. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

which may prove fatal (Norwood-Galloway et al. 1987).

Sclerema Neonatorum

This condition holds a grave prognosis for the infant. It is seen in premature and debilitated neonates, often with congenital heart disease, sepsis, or other severe illness (Kellum et al. 1968). The skin is diffusely involved with a wax-like hardening comparable to that of the cadaver. The skin including the fat is edematous with hypertrophied adipocytes showing triglyceride crystal formation within their cytoplasm. Fibrous bands are present, but there is only rarely any inflammatory infiltrate. The child generally dies from the associated condition. The cause may be related to immature enzyme pathways allowing fat crystallization, and the reduced incidence of the disorder is attributed to the use of incubators.

Birthmarks

A birthmark or nevus is a cutaneous lesion present at birth that may persist, enlarge, or regress with age. The terminology is confusing, but any form is often prefaced by either a cell type (melanocytic), or the tissue formed (vascular, lymphatic, organoid), or the epithelium involved (epidermis).

Pigmented Birthmarks

Mongolian Spot

The blue lesion presents over the sacrum and back in approximately 10% of white and up to 95.5% of black babies and tends to regress in childhood (Osburn et al. 1987; McLaurin 1988; Nanda et al. 1989; Gross et al. 1990). The diameter may extend from a few millimeters to several centimeters. Widely scattered collections of pigmented, dendritic melanocytes are located deep in the dermis, supporting the suggestion that migration arrest occurred during development.

Café-au-Lait Spots

These spots are well-circumscribed and uniformly pale brown lesions, one or more of which can be found in 0.8% of newborn white babies and in 12% of black babies. Children have higher numbers, probably because pigmentation is less developed in the neonate (Sigg et al. 1990). The majority occur in normal children, but just occasionally they are associated with systemic disorders such as type I neurofibromatosis or Albright's syndrome. In the former, Crowe and Schull (1953) have suggested that six or more lesions greater than 1.5 cm in diameter in a child is presumptive evidence of neurofibromatosis. In Albright's syndrome, a single large café-au-lait spot with an irregular edge is usually encountered. There is increased epidermal pigmentation without an increase in melanocytic cells.

Lentigines

These dark brown or black lesions are usually acquired during life in sun-exposed sites (solar lentigines). However, they may be present at birth, especially with certain systemic disorders, for example lentigines of the oral mucosa and lips in Peutz–Jeghers syndrome. Gorlin et al. (1969) described a syndrome with autosomal dominant inheritance and multiple lentigines that is known by the mnemonic LEOPARD: *l*entigines, *e*lectrocardiographic conduction defects, *o*cular hypertelorism, *p*ulmonary stenosis, *a*bnormalities of genitalia, *r*etardation of growth, and *d*eafness. The

NAME or LAMB or myxoma syndrome (*l*entiginous *n*evus; *a*trial or *m*ucocutaneous *m*yxomas, *m*yxoid neurofibromas, *e*phelides and *b*lue nevi) is a further syndrome, and lentiginosis profusa, speckled lentiginous nevus, and nevus spilus are other clinical examples apparent from birth (Fulk 1984).

Congenital lentigines have increased numbers of melanocytes disposed singly along the base of the epidermis associated with increased pigmentation of the basal epidermal cells and elongation of the rete pegs.

Melanocytic Nevi and Malignant Melanoma

Melanocytic nevi are formed by melanocytes and can involve the epidermis or the dermis, possibly reflecting their progress along the melanocytic development pathway (Cramer 1988). Clinically, congenital and acquired forms are recognized, but histologically this distinction is not always possible (Rhodes et al. 1985; Nickoloff et al. 1986; Cramer 1988) and may in fact be false, as in some congenital lesions pigmentation has not developed at birth (Osburn et al. 1987). Consequently, underreporting of incidence figures may result (Osburn et al. 1987; Sigg et al. 1990) and, more importantly, estimates of malignant potential (Rhodes 1986; Gari et al. 1988).

Melanocytic nevi are present at birth in approximately 1% of white babies (Jacobs and Walton 1976; Rhodes and Melski 1982; Gross et al. 1990) and grow at the same rate as the child (Rhodes 1986). They vary in size (Fig. 30.11) from tiny lesions to giant hairy pigmented lesions. The latter may show a dermatomal distribution on the trunk, with sparing of the distal extremities (garment or bathing trunk nevi). Such nevi (i.e., greater than 10 cm in diameter) occur in fewer than 1 in 20,000 neonates, and may be associated with leptomeningeal melanosis (Slaughter et al. 1969).

Small melanocytic lesions are junctional or compound nevi histologically (Jacobs and Walton 1976). The larger lesions tend to involve the reticular dermis and subcutis, with nevus cells sometimes growing in an Indian file or neurofibroma-like pattern (Mark et al. 1973). A study has concluded that congenital nevi cannot be reliably distinguished histologically from acquired nevi, apart from deep and large lesions (Cribier et al. 1999).

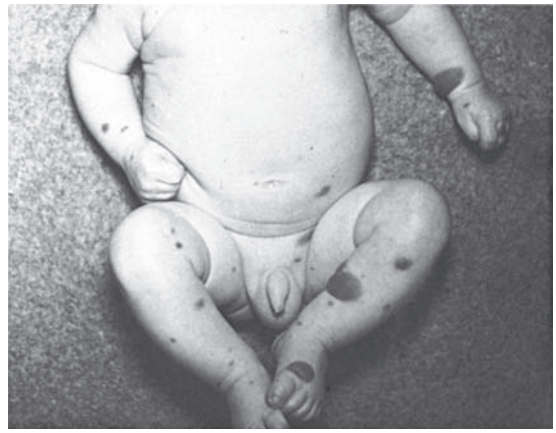


Figure 30.11. Multiple congenital pigmented nevi. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

Malignant melanoma is extremely uncommon in childhood. Melanoma encountered in the neonatal period has followed transplacental spread from the mother (Kruisinski and Saurat 1989), rather than in relation to a melanocytic nevus. Aside from these cases, three major patterns have been described: small blastic cell melanomas that have a high mortality rate, those resembling adult melanomas, and Spitzoid tumors (Barnhill 1998). Giant nevi have a recognized malignant potential (Gari et al. 1988; Marghoob et al. 1996), with the lifetime probability of developing melanoma estimated at 6.3%, 16.6 times the risk to a normal person (Rhodes 1986). Small melanocytic nevi may also carry a risk of malignant change (Swerdlow et al. 1995). Rhodes and Melski (1982) examined 134 patients with melanoma and found that 15% described a pigmented lesion present since birth. Examination of 234 melanomas revealed a melanocytic nevus in contiguity with melanoma in 64, and 19 of these patients described a congenital nevus. In a recent study of melanomas associated with nevi, eight of 46 nevi showed the same allelic deletions on chromosome 9p21 as their associated malignant melanoma, consistent with a causal relationship (Bogdan et al. 2003).

Other Melanocytic Birthmarks

A rare congenital melanocytic lesion is the nevus of Ota, usually seen in female Asian children. There is unilateral patchy discoloration in the

region of the second division of the trigeminal nerve (Rhodes 1983). Two thirds of patients have involvement of the sclera, and malignancy can develop (Dompmartin et al. 1989). The nevus of Ito is similar but is more extensive, also involving the shoulder. Dermal melanocyte hamartoma is a diffuse variant (Burkhart and Gohara 1981). The histological features of these lesions are essentially identical, showing pigmented dendritic melanocytes that are more numerous and more superficially placed than in Mongolian spots.

Congenital Vascular Abnormalities

Lesions present at birth or shortly thereafter that are due to a vascular ectasia or proliferation are quite common. Of these, the most frequent are capillary or strawberry hemangiomas (Osburn et al. 1987). The distinction, often made, between capillary and cavernous hemangiomas is not helpful histologically, since the two merge and often cannot be clearly distinguished. A few examples are associated with clinical syndromes recognizable at birth. These include the blue rubber-bleb nevus syndrome, in which there are also hemangiomas in the gastrointestinal tract and other organs; diffuse neonatal hemangiomatosis, where multiple skin hemangiomas are accompanied by widespread similar visceral lesions; and Maffucci's syndrome, in which the skin lesions are associated with multiple enchondromas.

Strawberry Nevus

Present in 1.1% to 2.6% of neonates and seen in 10% of children between the ages of 1 month and 1 year, strawberry nevi are raised deep-red lesions that invariably regress spontaneously (Fig. 30.12), especially the smaller examples (Salmon patches). They may be heralded at birth by a flat area of telangiectasia that rapidly changes into a typical lesion. This change follows canalization between ectopic islands of vascular tissue and the dermal vessels. Any part of the body may be affected. Most are less than 5 cm in size, but occasionally very large examples may result in significant arteriovenous shunting. Similar and sometimes fatal shunting can complicate diffuse hemangiomatosis (Held et al. 1990).

These nevi consist of a lobular proliferation of ectatic blood-filled vascular spaces lined by endothelial cells. Ectatic vessels in the deep dermis or subcutaneous tissue may have smooth muscle fibers in their walls, and those with a significant deep component may resemble cavernous hemangiomas (Figs. 30.13 and 30.14). Perineural invasion may be seen, but this is a benign feature (Calonje et al. 1995).

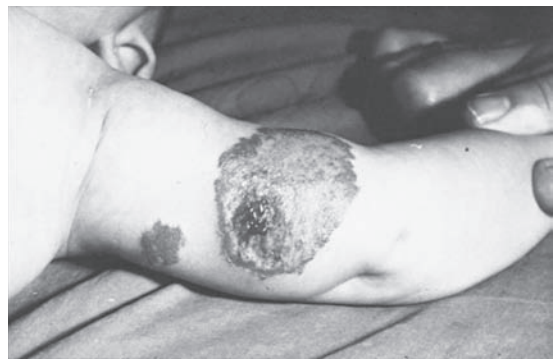


Figure 30.12. Capillary hemangiomas on the arm of an infant. The larger lesion shows surface ulceration. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

Regression is seen as a blue discoloration of the overlying skin, and histologically there is thrombosis and fibrosis of the ectatic vessels culminating in scar tissue. By the age of 7 years, 70% of hemangiomas have regressed. The smallest lesions (salmon patches) found in 44% of neonates of either sex can all be expected to have disappeared by 6 years of age. Those at the nape of the neck (stork bite mark) will take the longest time (Leung and Telmesani 1989).

Port Wine Stain

This flat pink lesion is present in 0.3% of neonates. It does not involute but grows with the child. It is often unilateral and may follow a dermatomal distribution. There is no histological abnormality in the neonate, but in an older child dermal capillaries are dilated but not increased in number (Jacobs 1983; Finlay et al. 1984).

A port wine stain in the distribution of the first division of the trigeminal nerve may be a manifestation of the Sturge–Weber syndrome. Concurrent, ipsilateral leptomeningeal angiomatosis may

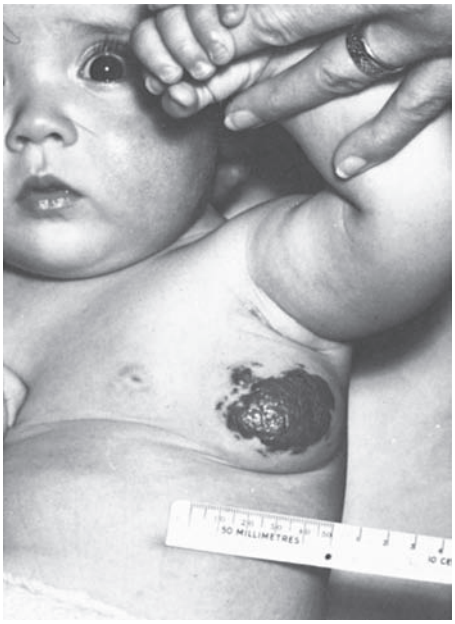


Figure 30.13. Cavernous hemangioma. There is soft tissue involvement extending beyond the margin of the superficial skin lesion.

result in focal seizures. Approximately 45% of babies also have ipsilateral congenital glaucoma, indicating involvement of the maxillary and ophthalmic divisions of the trigeminal nerve.

Angiokeratoma

Angiokeratomas are present in a variety of different clinical conditions, including angiokeratoma circumscriptum, angiokeratoma of Mibelli, solitary angiokeratoma, angiokeratoma of Fordyce (scrotal), and angiokeratomacorporis diffusum. Only angiokeratoma circumscriptum is present from birth. The histology is identical in all varieties, comprising numerous thin-walled, ectatic blood vessels in the papillary dermis, and underlying epidermis that demonstrates a variable degree of hyperkeratosis and acanthosis (Fig. 30.15).

Lymphangiomas

These malformations are characterized by dilated lymphatic vessels lined by endothelium. Distinction from hemangiomas is not always easy, since bleeding into some occurs, and a good antibody marker for lymphatic endothelium has not been developed, although lymphangiomas are generally negative for factor VIII–related antigen (Burgdorf et al. 1981). Lymphangioma circumscriptum is the commonest form in the neonate. It is present at birth or develops in early infancy and persists, with a predilection for neck, shoulders, proximal limbs, perineum, and mouth. Multiple small vesicles are seen on one or more circumscribed areas of the skin filled with clear or blood-stained fluid. The vesicles are ectatic lymphatic spaces within the papillary dermis, and deep to this are dilated lymphatics, some with smooth muscle in their walls (Fig. 30.16). The high incidence of recurrence after excision, together with lymphangiographic observations, led Whimster (1976) to propose that the primary abnormality was one or more lymph cisterns deep in the subcutis and not in continuity with the main lymphatic system.

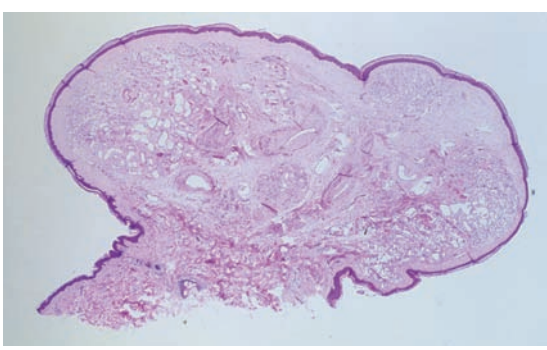


Figure 30.14. Cavernous hemangioma with subcutaneous and dermal vessels; those more deeply placed have thicker walls containing smooth muscle (×9).

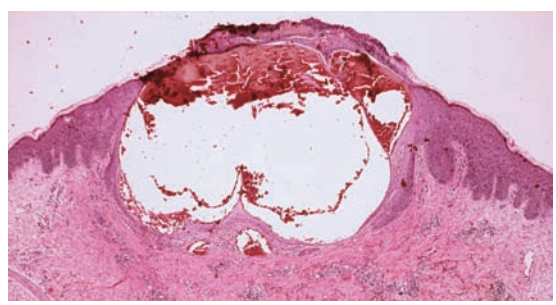


Figure 30.15. Angiokeratoma. Ectatic blood vessels in the papillary dermis with overlying parakeratosis and adjacent epidermal acanthosis. (×40)

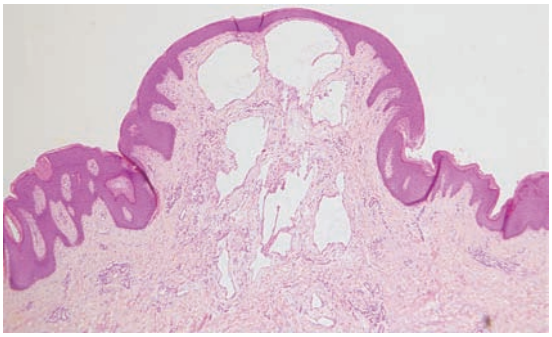


Figure 30.16. Lymphangioma. Dilated empty lymphatics are present within the papillary and reticular dermis.

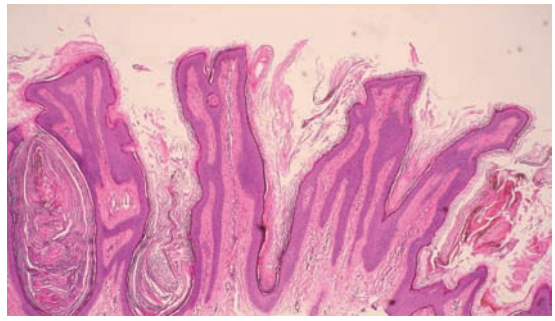


Figure 30.17. Epidermal nevus. The epidermis shows boxy papillomatosis, with hyperkeratosis and variable acanthosis. (×200)

The cisterns give rise to the dermal ectatic vessels, and are not affected by superficial excision.

Another type (which may have a similar histogenesis) is the cavernous lymphangioma or cystic hygroma. This comprises one or more large cystically dilated spaces in the dermis, subcutis, or intermuscular septum. They tend to occur on the neck or in the axilla, groin, or popliteal fossa.

Other Birthmarks

Epidermal Nevi and Organoid Nevi

Localized malformations of the epidermis or skin appendages had an incidence of 0.2% in one neonatal survey (Jacobs and Walton 1976). The epidermal nevus appears darker than the surrounding skin, can occur anywhere on the body, has a warty or granular surface, and may be single and localized or linear and extensive. Sometimes spiral streaks are seen. The epidermal nevus is papillomatous and hyperkeratotic with irregular acanthosis, frequently with a boxy appearance (Fig. 30.17). Extensive bilateral epidermal nevi may mimic the histology of epidermolytic hyperkeratotic ichthyosis, and in such a case is referred to as ichthyosis hystrix (Hurwitz 1983) (Fig. 30.18).

The organoid or sebaceous nevus characteristically occurs on the scalp as a flat, yellow–pink, hairless plaque. There is hyperkeratosis, irregular acanthosis, and papillomatosis; skin appendages are malformed and irregular. At puberty apocrine glands are a prominent feature. During a lifetime, 10% to 15% of organoid nevi are said to undergo neoplastic proliferation, usually giving rise to basal cell carcinoma and occasionally to syringocystadenoma papilliferum or other adnexal, sometimes unclassifiable, tumors (Fig. 30.19).

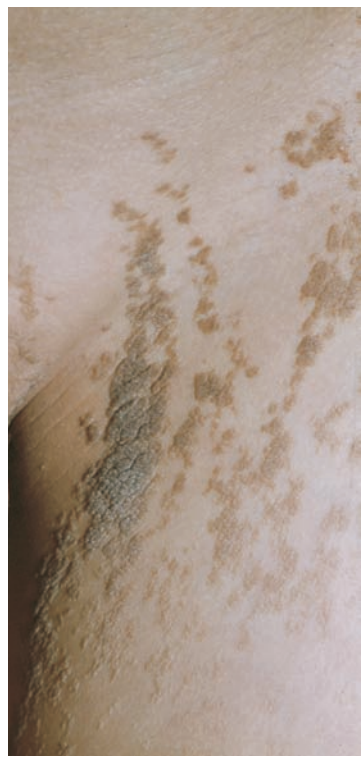


Figure 30.18. Ichthyosis hystrix. The lesion was initially diagnosed as an epidermal nevus but later recognized as ichthyosis hystrix. The microscopic features of the two conditions are extremely similar.

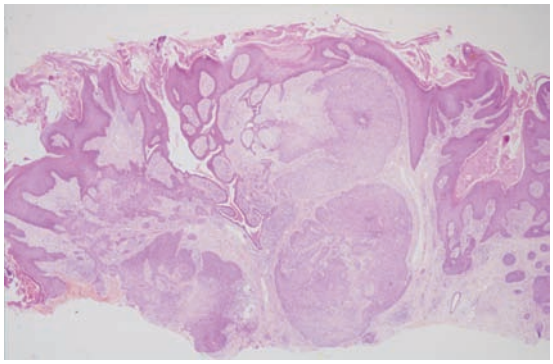


Figure 30.19. Sebaceous nevus including syringocystadenoma papilliferum. In the center is a duct with an apocrine lining and surrounding this are islands of poorly differentiated sebaceous gland cells and undifferentiated cells.

Congenital Infections with Skin Manifestations

Viral Infections

Viral infections may be transmitted across the placenta, from the birth canal or from lesions on the mother or medical staff. The diagnosis may be reached from examination of neonatal and maternal tissues including serum, and it can be aided in biopsy material by labeled antibodies and molecular biology techniques. Not all such viral infections declare themselves in the neonatal period, most notably human immunodeficiency virus (Straka et al. 1988; Lim et al. 1990). Others, such as cytomegalovirus, manifest themselves in the neonate in different forms from those found in older infants (Sandler and Snedeker 1987).

Cytomegalovirus and Rubella

Congenital infections such as cytomegalovirus (CMV) and rubella sometimes exhibit a generalized rash at birth that is maculopapular and purpuric, and occasionally includes blueberry-muffin lesions; these are magenta, 2 to 7 mm, have a raised, indurated character, and persist for 3 to 6 weeks. They comprise foci of erythrocyte precursors within the dermis and subcutis, especially around eccrine adnexae. As dermal erythropoiesis is normal in embryos with a crown–rump measurement of less than 50 mm, these infections are thought to be due to persistence of erythroblastic elements rather than an infiltrative process (Brough et al. 1967). Similar lesions are occasionally seen in other viral disorders in the neonate, as well as in congenital toxoplasmosis (Kruisinski and Saurat 1989).

Herpes Viruses

Congenital herpes simplex virus (HSV) infections are usually due to type II virus, and acquired from active maternal genital disease prior to seroconversion. Occasionally, transplacental spread of virus, type I or II, occurs, and a few infections have resulted from home or hospital contacts (Francis et al. 1975). Over half of affected neonates have vesicles on the scalp and face erupting between the fourth and tenth days of life. There may be conjunctivitis, or generalized eruption. Systemic disease follows rapidly, and examples can be expected to increase because of the rising prevalence of HSV type II infection (Arvin and Prober 1997). Histological diagnosis is usually straightforward, classically demonstrating acantholysis of ballooned, multinucleated keratinocytes. Immunohistochemical stains may also assist in the diagnosis.

Congenital varicella syndrome, in which infection occurs in the first trimester, includes abnormalities of limbs, eyes, brain, and skin. Skin lesions heal, leaving scars at birth. Hyperplasia or absence of digits is sometimes observed. Varicella infection acquired shortly before delivery resembles postnatal infection (Fig. 30.20), but hemorrhagic pneumonitis is a serious and sometimes fatal complication in the neonatal period.

Bacterial Infections

Group A β-Hemolytic Streptococcus

Neonates may acquire the highly contagious impetigo, which is caused by a group A β-hemolytic streptococcus. Predisposing factors include a warm humid atmosphere and poor hygiene. Infants are also at risk of streptococcal cellulitis or erysipelas, which is a rapidly spread-

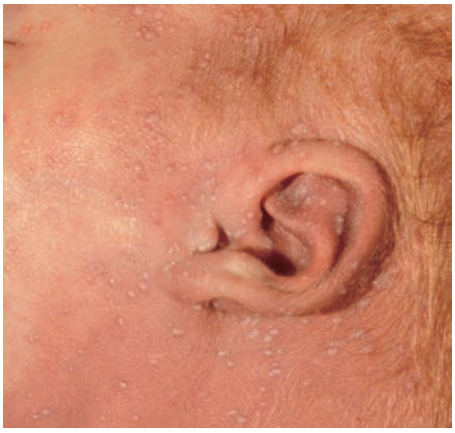


Figure 30.20. Congenital chickenpox. Infection was acquired late in gestation. Multiple small papules and vesicles are present over the face and scalp.

ing superficial infection of skin precipitated by minor trauma, including needle puncture and circumcision.

Staphylococcus aureus

Bullous impetigo is due to *Staphylococcus aureus* and may be passed to a neonate from an attendant carrier producing infection in the nappy area or umbilicus. The causative organism is a phage group II staphylococcus, most commonly type 71 (Tunnessen 1983).

Staphylococcus is the commonest organism to colonize the devitalized umbilical cord stump. Omphalitis and occasionally cellulitis of the abdominal wall may result (Fig. 30.21).

Staphylococcal Scalded Skin Syndrome

This syndrome affects neonates, infants, and small children, and is due to an epidermolytic exotoxin produced by phage group II staphylococci. An affected baby may appear well despite an extensive erythematous exfoliative rash with a predilection for the face, upper trunk, and groin areas (Fig. 30.22). There is a split in the epidermis at the level of the granular layer, often with occasional acantholytic cells, without a significant inflammatory infiltrate. The staphylococci producing the toxin are often found in the pharynx (Elias et al. 1977; Hansen 1983).

Congenital Syphilis

This disorder continues to appear and may be increasing. Affected neonates can be seronegative at birth despite positive serology in the mother, and both baby and mother can be negative at the time of the birth (Dorfman and Glaser 1990). The skin involvement is most usually a diffuse macular papular eruption but atypical presentations are seen and in all examples there is systemic involvement. If there is a biopsy, demonstration of treponema with a silver preparation confirms the diagnosis.

Congenital Genetically Determined Diseases

Disorders of Keratinization

The ichthyoses are a group of clinical disorders characterized by abnormalities of keratinization that are mainly expressed in the epidermis but

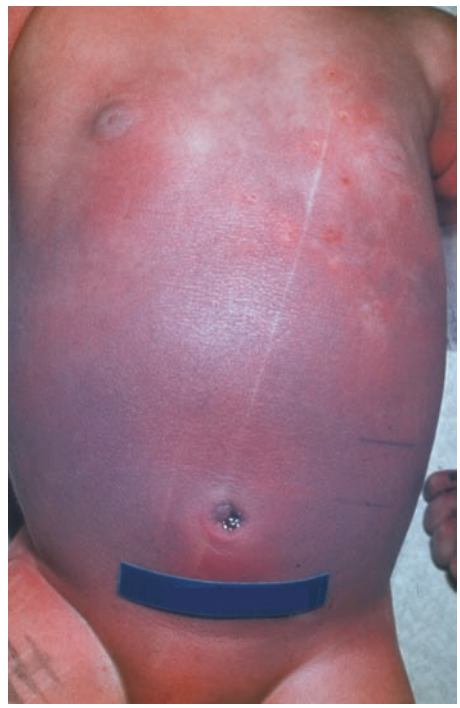


Figure 30.21. Omphalitis has progressed to cellulitis involving most of the abdominal wall. (Courtesy of Dr. P.S. Andrews, Kettering, England.)

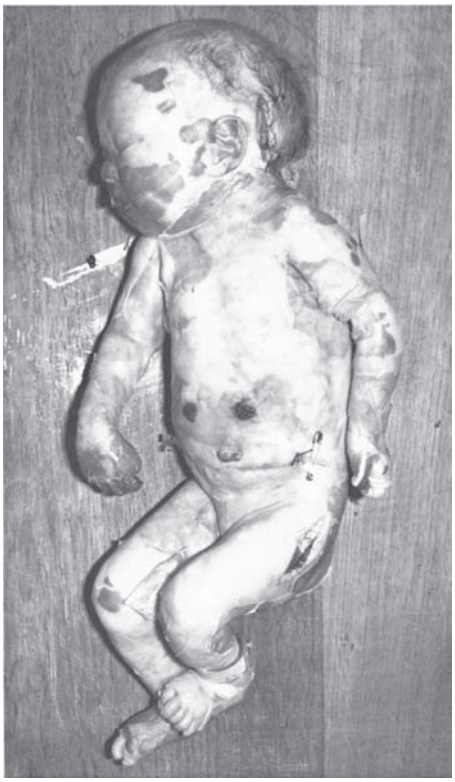


Figure 30.22. Staphylococcal scalded skin syndrome. Exfoliation of the epidermis affecting the face, shoulders, hands and feet. (Courtesy of Dr. A. King, Cambridge, England.)

that can also be manifested in mucous membranes. Patients usually have a thickened skin with prominent scales often compared to those of fish, and a distribution pattern that aids in subclassifying the disorder. Well-recognized variants, particularly among neonates, include those with a bullous component (epidermolytic hyperkeratosis) and those with erythroderma (epidermolytic hyperkeratosis and nonbullous ichthyosiform erythrodermia) (Table 30.2) as well as an increasing number of associated syndromes with either neuroectodermal or mesodermal defects (Table 30.3). The morphological features overall are slight, and interpretation depends on ancillary clinical detail and sometimes ultrastructural studies.

An effect of molecular studies on ichthyosis has been to bring to light molecular abnormalities that are similar to those in clinically divergent conditions, for example, many blistering disorders. Classifications are consequently in a state of flux and may change from the predominantly clinical approach to a pathogenic basis. The abnormal keratin expression common to some forms of ichthyosis and blistering disorders has resulted in some texts in all of these states being grouped as disorders of keratinization. Within this text a clinically biased classification is maintained.

Collodion Baby

At birth, a baby may be covered by tight shiny collodion-like membrane (Fig. 30.23). Within a few days this membrane detaches, leaving the underlying skin red and scaly. The collodion membrane consists of orthokeratin (De Dobbeleer et al. 1982). Most collodion babies have lamellar ichthyosis, but occasionally the subsequent clinical picture resembles X-linked ichthyosis or ichthyosis vulgaris.

Lamellar Ichthyosis

Lamellar ichthyosis presents at birth either as a collodion baby or with generalized large epidermal scales 0.5 to 1.5 cm in diameter (Figs. 30.24 and 30.25).

There is moderate thickening of the epidermis, uniform marked hyperkeratosis with focal parakeratosis, a prominent granular layer, moderate papillomatosis, and a mild patchy chronic inflammatory infiltrate within the dermis (Frost and van Scott 1966). Keratinosomes are prematurely formed, and synthesis of keratohyaline granules within the stratum spinosum is reduced. Nucleated cells and cell organelles are seen high in the granular layer. Hyperkeratosis may be due to a combination of the increased epidermal proliferation, abnormal keratin formation, and increased adhesiveness of the upper epidermis, possibly mediated by keratinosomes.

The term *lamellar ichthyosis* embraces a heterogeneous group of abnormalities with two distinct subtypes, although both show mutations in the same three genes (Akiyama et al. 2003). The first, lamellar ichthyosis, has the histological features described above; the second, nonbullous ichthyosiform erythroderma, has more marked

Table 30.2. Congenital disorders of keratinisation in the neonate

Name	Genetics	Present at birth	Frequency	Histopathology	Turnover	Histogenesis	References
X-linked ichthyosis	X-linked recessive	Rarely Collodion baby	1:6000	Hyperkeratosis. Granular layer normal or slightly thick. Epidermis slightly thick	Normal	Steroid sulphatase deficiency	Epstein et al. (1981) Shapiro et al. (1978) Rehfield et al. (1988)
Lamellar ichthyosis	Autosomal recessive	Usually Collodion baby or generalised ichthyosis	Rare 1:3,000,000	Hyperkeratosis. Normal or thick granular layer Moderate acanthosis	Increased	Increased intercellular cement Reduced synthesis of keratohyaline granules	Vandersteen and Muller (1972)
Epidermolytic hyperkeratosis (bullous ichthyosiform erythroderma)	Autosomal dominant	Generalized erythema Blisters	Rare <1:100,000	Hyperkeratosis. Thick epidermis. Vacuolation of stratum spinosum and granular layer	Increased	Premature keratohyalin granules. Clumped tonofilaments interfering with desmosomes	Anton-Lamprecht (1983)
Harlequin fetus	Autosomal Recessive	Thick plates of stratum corneum	Very rare	Variable; ortho- and sometimes parakeratosis	Unknown	Stratum corneum proteins abnormal	Baden (1982) Dale et al. (1990)
Non-bullous ichthyosiform erythroderma	Autosomal recessive	Generalized ichthyosis; fine scales and erythema Collodion baby	Rare	Prominent acanthosis Moderate hyperkeratosis. Focal parakeratosis	Increased	Unknown	Williams and Elias (1985) Hazell and Marks (1985)

Table 30.3. Syndromes associated with ichthyosis (Williams and Elias 1987)

CHILD	Hemidysplasia of limbs
Conradi	Skeletal; ocular
IBIDS	Brittle hair; impaired IQ; decreased fertility; short stature
KID	Deafness
Netherton	Trichorrhexis invaginatum
PIBIDS	Photosensitivity; IBIDS features
Refsum	Cerebellar ataxia; progressive paresis; retinitis pigmentosa
Rud	Hypogonadism; mental deficiency; epilepsy
Sjögren-Larsson	Mental retardation; spastic paresis

CHILD, congenital hemidysplasia with ichthyosiform erythroderma and limb defects; IBIDS, impaired IQ, brittle hair, decreased fertility, short stature; KID, keratitis, ichthyosis, deafness; PIBIDS, photosensitivity + IBIDS features.

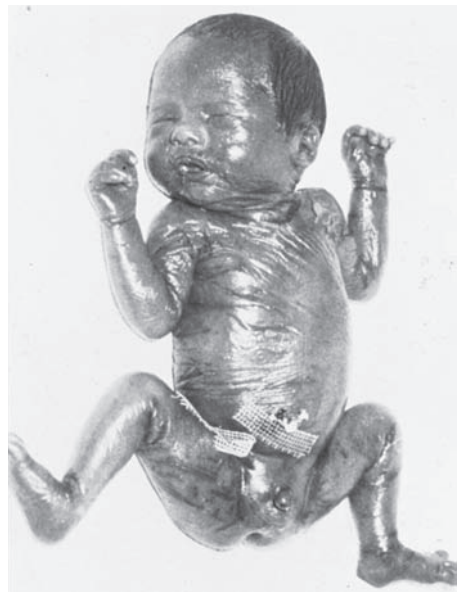


Figure 30.23. Collodion baby. There is a tight shiny membrane involving the entire surface. (Courtesy of R.M. Marks, Cardiff, England.)

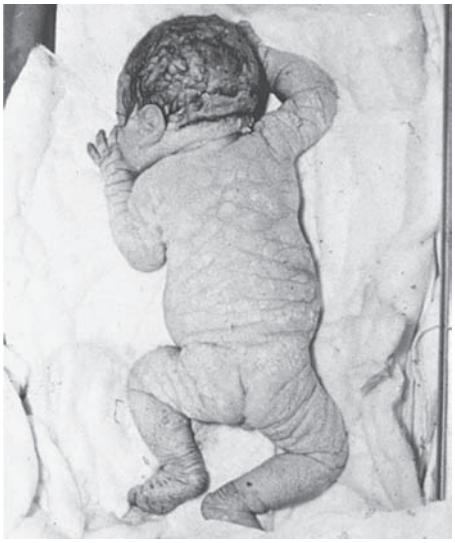


Figure 30.24. Lamellar ichthyosis. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

acanthosis and only moderate hyperkeratosis, more rapid cell turnover, and focal parakeratosis. Epidermal scales are smaller and finer than classic lamellar ichthyosis, and erythroderma is common (Hazell and Marks 1985; Williams and Elias 1985). The combination of associations with neutral fat storage disorders and abnormalities of lamellar bodies have led to suggestions of an underlying enzyme defect (Williams 1992).

Harlequin Fetus

Harlequin fetus is a very rare recessive disorder in which the neonate is covered with thick plates of stratum corneum at birth. Affected babies usually die from sepsis within the first 2 weeks of life. A range of histological abnormalities is described, from hyperkeratosis with a normal granular layer to parakeratosis with an absent granular layer. Different x-ray diffraction patterns between patients suggests that the clinical presentation comprises more than one histogenetic entity (Baden 1982; Dale et al. 1990). Impaired lamellar body formation and function with consequent defective proteases has been postulated as the basis of the hyperkeratosis (Milner et al. 1992).

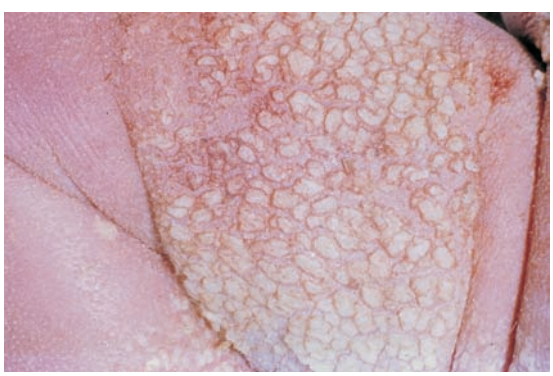


Figure 30.25. Lamellar ichthyosis. High power of skin on Figure 30.23. The scaly nature of the skin is apparent, an appearance termed "icing sugar" skin.

X-Linked Ichthyosis

At birth X-linked ichthyosis is only occasionally present as generalized mild scaling, more rarely as a collodion baby. Ninety percent of cases have an X-linked gene deletion that is heterogeneous in the female infants and rarely associated with male infants, probably because they die before birth. The disease is due to a deficiency of steroid sulfatase in fetal epidermal cells; cultured fibroblasts and placental leukocytes also show the defect (Shapiro et al. 1978). The result is a high concentration of cholesterol sulfate in the stratum corneum and hyperkeratosis (Honour et al. 1985; Rehfield et al. 1988).

Epidermolytic Hyperkeratosis

Alternatively known as congenital bullous ichthyosiform erythroderma, epidermolytic hyperkeratosis is a rare autosomal dominant condition that may present at birth with generalized erythema, vesicles, and bullae, with subsequent development of thick brown scales. Localized forms sometimes resemble linear nevi; indeed, epidermolytic epidermal nevi appear to be a mosaic disorder with the same genetic defect as epidermolytic hyperkeratosis (Paller et al. 1994). There is inter- and intracellular edema within the upper stratum spinosum and stratum granulosum, a thick granular layer with irregular keratohyalin granules, and hyperkeratosis (Frost and van Scott 1966) (Figs. 30.26 and 30.27). Cell turnover is increased. The tonofibrils of the suprabasal epidermal cells are clumped, thereby reducing the effectiveness of desmosomal junctions, resulting in separation and bulla formation.

In keeping with this is a mutation of keratin genes *K1* and *K10* in the carboxyl terminal of the rod domain of *Keratin 1* and the amino terminal of that of *Keratin 10* (Rothnagel et al. 1992). Anton-Lamprecht (1983) suggests that the increased cell turnover and hyperkeratosis are secondary reaction mechanisms.

Palmoplantar Keratodermas

Palmoplantar keratodermas form a heterogeneous group of congenital and acquired disorders of keratinization mainly seen on the palms and soles but sometimes diffusely distributed. Ectodermal abnormalities are often associated, and at least 10 hereditary, dominant and recessive, forms are described, many evident at birth (Sybert et al. 1988). Mutations have been found in a number of genes primarily concerned with the correct functioning of the cornified cell envelope (Kimyai-Asadi et al. 2002). The histological changes range from epidermal thickening, including the granular layer, with hyperkeratosis, to appearances similar to those in epidermolytic hyperkeratosis (see above) and, in localized forms, thickening combined with deep dermal pitting of the epidermis.

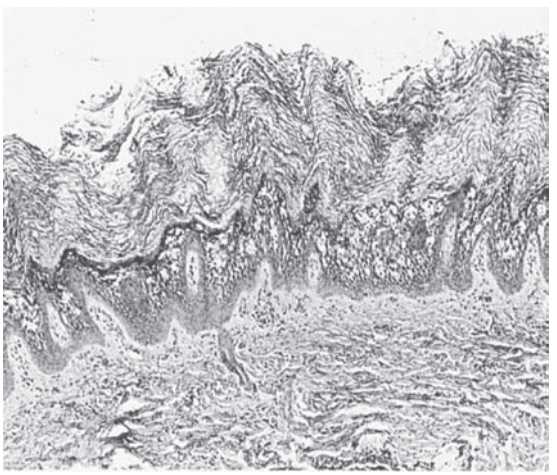


Figure 30.26. Epidermolytic hyperkeratosis. There is uniform hyperkeratosis, vacuolation within the upper epidermis and irregular clumping of keratohyalin granules. (Courtesy of Dr. J. Mahood, Northampton, England.)

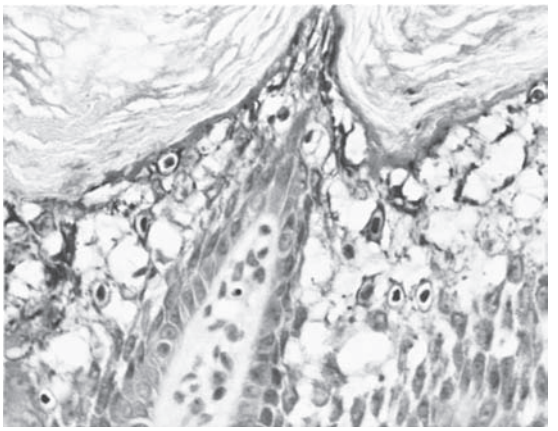


Figure 30.27. Epidermolytic hyperkeratosis. (Courtesy of Dr. J. Mahood, Northampton, England.)

Blistering Disorders

A wide range of blistering disorders is encountered in neonates, although fortunately most are uncommon and innocuous (Frieden 1989). The disorders may be subdivided clinically into overlapping groups, including those in which the main lesions are pustules or vesicles (predominantly infective conditions); bullae (sucking blisters, epidermolysis bullosa, etc.); and erosive ulcerated lesions (staphylococcal scalded skin, toxic epidermal necrolysis, etc.). A fairly common finding is that the distribution of the lesions is often to those areas most exposed to mechanical stress, such as the shoulders, elbows, and heels. The infective conditions have the distinction that therapy is often available and that diagnosis can be made from appropriate swab and culture specimens. The much rarer genetic causes often require electron microscopy and immunocytochemistry or immunofluorescent mapping (Fine et al. 1987) to define the level of blister formation in the dermoepidermal junction, for example, epidermolysis bullosa (Table 30.4), while others involve biochemical investigations, for example, acrodermatitis enteropathica (zinc deficiency) and erythropoietic porphyria. As a part of these studies, one can take advantage of the effect of mechanical stress and produce a blister by gentle rubbing prior to biopsy.

Table 30.4. Neonatal forms of epidermolysis bullosa

Name	Genetics	Present at birth	Incidence	Level	Antigen mapping		Electron Microscopy
					Blister base	Blister roof	
Non-scarring							
Epidermolysis bullosa simplex	Autosomal dominant	Sometimes	1:50 000	Intraepidermal	BPA Laminin Type IV/VII collagen		Vacuolation of basal cells
Junctional epidermolysis bullosa (Herlitz)	Autosomal recessive	Yes	Very rare	Junctional	Laminin Type IV/VII collagen	BPA	Decrease and hypoplasia of basal hemidesmosomes
Scarring							
Dominant dystrophic epidermolysis bullosa	Autosomal dominant	Usually late infancy	1:100 000 to 1:50,000	Subepidermal	Type VII collagen	BPA Laminin Type IV collagen	Reduction of anchoring fibrils
Recessive dystrophic epidermolysis bullosa	Autosomal recessive	Yes	1:3,000,000	Subepidermal			Absence of anchoring fibrils

Eady 1987; Rasmussen et al. 1989. BPA: Bullous pemphigoid antigen.

Epidermolysis Bullosa Simplex

Epidermolysis Bullosa Simplex is the commonest form of epidermolysis bullosa. Blistering occurs in the first few minutes of life from gentle trauma and is most often seen on the extremities (Fig. 30.28). Mucous membrane involvement may occur, and blisters heal without scarring. The disorder improves before adolescence. Mutations in the keratin 5 and 14 genes underline the condition (Bonifas et al. 1991), and the tonofibril clumping is evident ultrastructurally (Moss and Savin 1995).

Junctional Epidermolysis Bullosa

Neonates with this autosomal recessive condition are born with erosions and bullae, and minimal trauma promotes blistering (Fig. 30.29). A similar tendency for mucosal separation is seen in the gastrointestinal, genitourinary, and respiratory tracts (Schachner et al. 1977). The disease is divided into Herlitz or non-Herlitz forms, the former being more severe. Although the prognosis is poor, with death from sepsis usual in the first years, survival into adulthood is becoming possible with the use of corticosteroids. Pregnancy and oral contraceptive agents are also described as beneficial. A diagnosis can be made prenatally by means of a fetal skin biopsy. If the basal epidermal hemidesmosomes are normal by 18 to 20 weeks' gestation, junctional epidermolysis bullosa can be excluded (Anton-Lamprecht 1983). Molecular analysis can also contribute; most commonly, there are mutations of the kalinin/laminin 5 genes, which are associated with lack of hemidesmosome-associated protein in the basement membrane (Moss and Savin 1995).

Dystrophic Epidermolysis Bullosa

The bullae are large and they rupture, leaving deep ulcers (Fig. 30.30) that heal by scar formation. Autosomal dominant and recessive subtypes

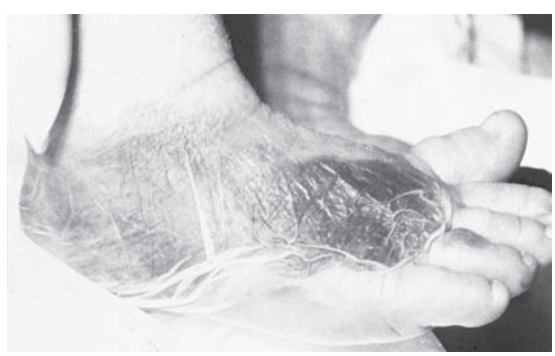


Figure 30.28. Epidermolysis bullosa, nonscarring. There is loss of the epidermis over the foot. (Courtesy of R.M. Marks, Cardiff, England.)

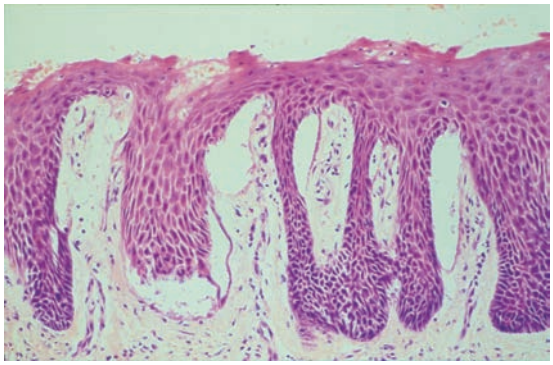


Figure 30.29. Junctional epidermolysis bullosa. The epidermis is thickened with elongated rete pegs and separation of the epidermis from the dermis.

are found, the disease being more severe in the recessive form with greater mucosal involvement, reduced life span, and an increased incidence of basal and squamous carcinoma of the skin.

Anton-Lamprecht and Schnyder (1973) reported an absence of anchoring fibrils in patients with dominant scarring epidermolysis bullosa. Briggaman and Wheeler (1975), using an elegant experiment involving recombination of epidermis and dermis from normal patients and patients with the recessive disorder, found that there is absence of dermal anchoring fibrils. Recombination of the abnormal epidermis with normal dermis resulted in the formation of anchoring fibrils, while the reverse did not. Recent observations confirm the absence of anchoring fibrils associated with the absence of type VII collagen (Bruckner-Tuderman et al. 1989; Rusenko et al. 1989) as well as chondroitin sulfate (Fine and Couchman 1989). Mutations of the type VII collagen gene (*COL 7AI*) underlie the condition, and recognition of these mutations in affected families may provide a means of prenatal diagnosis (Mellerio et al. 1997).

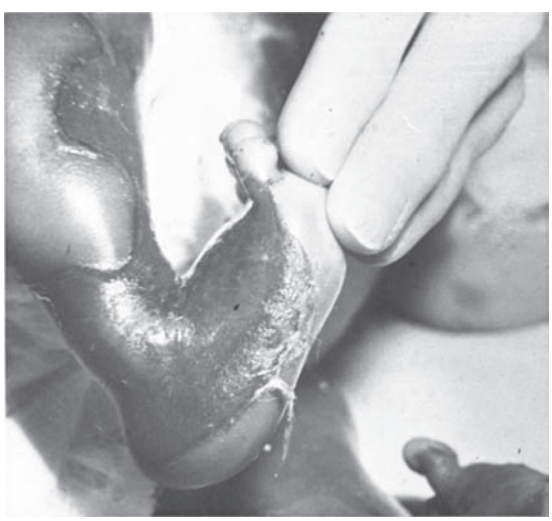


Figure 30.30. Dystrophic epidermolysis bullosa. Rupture of bullae leaves extensive, well-defined, deep ulcers. (Courtesy of Drs. T.J. Ryan and R. Dawber, Oxford, England.)

Other Genetic Diseases

Incontinentia Pigmenti

This rare disorder, almost exclusive to girls, is X-linked and dominant. Systemic changes including CNS, skeletal, dental, and particularly ocular abnormalities (Francois 1984) are invariably present and are the cause of morbidity. In a study of 455 patients with incontinentia pigmenti, 34 were blind at the time of examination (Carney 1976). The skin lesions occur as a series of stages, not always in the same sites of the body (Fig. 30.31). The first stage is present at birth or within the first days of life as linear erythematous vesicular lesions on the trunk and extremities. There may be a peripheral eosinophilia accompanying an interepidermal eosinophilic spongiosis, often with bulla formation (Fig. 30.32). The second stage occurs within weeks as verrucous growths, often on the extremities and characterized by acanthosis, papillomatosis, and hyperkeratosis. Later, these subside and linear whorls and streaks of pigmentation appear, especially on the trunk. At this stage melanin from melanocytes within the basal epidermis is present within dermal macrophages. All stages include dyskeratotic cells within the epidermis, some being phagocytosed by macrophages. Gradually the pigmented areas fade to leave atrophic or pigmented areas (Guerrier and Wong 1974; Carney 1976).

The pathogenesis is unknown, though Schamburg-Lever and Lever (1973) have suggested that dyskeratosis promotes macrophage migration into the epidermis, and incontinentia pigmenti results from subsequent macrophage elimination to the dermis. Basophils may stimulate the release of eosinophil chemotactic factor,

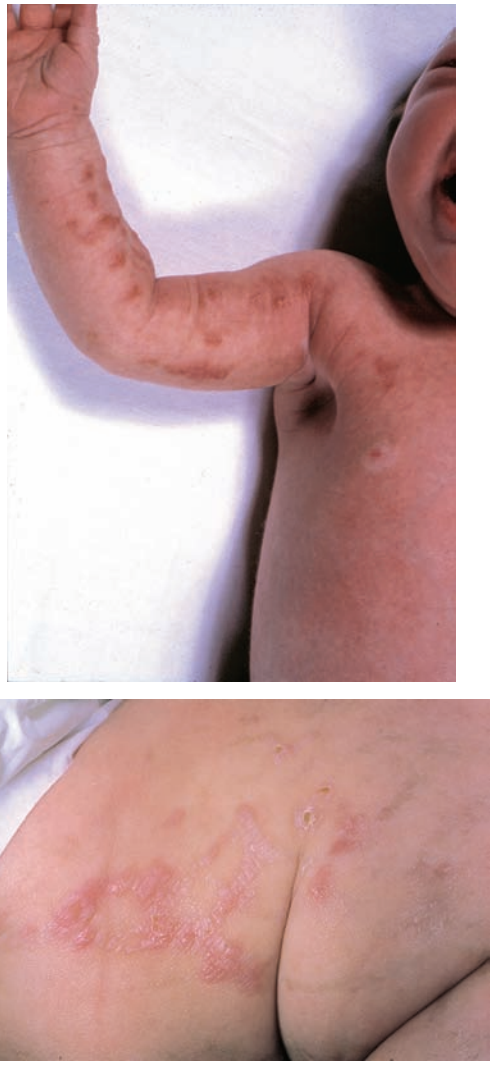


Figure 30.31. Incontinentia pigmenti. (A) Early lesions, which are reddened and vesicular. (B) Late lesions characterized by warty raised lesions.

which is evident in, and may be the cause of, the vesicle and bullous formation (Takematsu et al. 1986). The mutation has been localized to Xq28 (Smahi et al. 1994).

Darier's Disease (Keratosis Follicularis)

The linear or zosteriform type, which is limited to one side of the body, can present at birth, in contrast to the more usual presentations in adolescence and adulthood. The condition is autosomal dominant and caused by mutations in the *ATP2A2* gene, encoding an endoplasmic reticulum adenosine triphosphatase (ATPase), on chromosome 12 (Sakuntabhai et al. 1999). Suprabasal splitting with acantholysis, *corps ronds* and grains, as well as clefting and villi are characteristic (Burge and Wilkinson 1992).

Lipoid Proteinosis (Hyalinosis Cutis et Mucosae)

This autosomal recessive disorder can present at birth. Cutaneous manifestations involving waxy papular nodular lesions on the face, elbows, knees, and hands lead ultimately to focal scarring. Systemically, lesions principally occur on the tongue, oral mucosa, and vocal cords, and fits signify cerebral involvement. Microscopically, the characteristic finding is amorphous, periodic acid-Schiff (PAS)-positive eosinophilic deposits around vessels and, additionally, in the dermis and around sweat glands. These deposits include glycoproteins, and there is a decrease in dermal collagen, mainly collagen 1 (Owen et al. 1988). A mutation in the extracellular matrix protein 1 gene, on chromosome 1q21, has been identified (Hamada et al. 2002).

Skin Disorders with Disordered Immune Responses

The immune responses of the neonate are incompletely developed and are also affected by those of the mother. The effect of the latter will

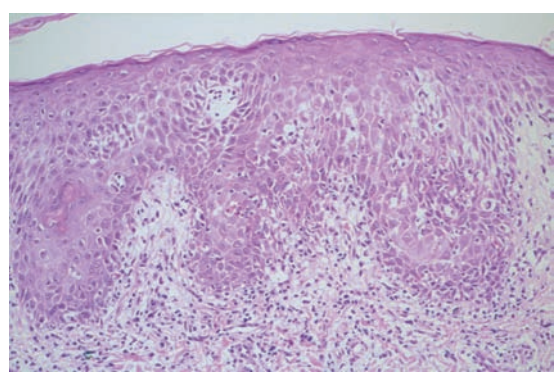


Figure 30.32. Incontinentia pigmenti. The earliest lesions include edema with vesicle formation with dyskeratotic cells in the dermis. This example includes very few eosinophil granulocytes.

be to mask the appearance of primary immunodeficiency states in the neonate and the appearance of skin disorders associated with such conditions. If, however, the mother's immune responses are abnormal, skin disorders may be transmitted to the fetus and some may be found in the neonate (Kruisinki and Saurat 1989). Herpes gestationis and pemphigus vulgaris are rare examples of disease caused by circulating maternal autoantibodies (Merlob et al. 1986; Shornick 1987). The routine treatment of many immunodeficiencies by bone marrow transplantation means that the most commonly encountered skin biopsies from immunodeficient children tend to be related to the assessment of graft versus host disease.

Neonatal Lupus Erythematosus

Although the mother has systemic lupus erythematosus, she may be asymptomatic during pregnancy, and thus the condition is unsuspected. Circulating Ro (SSA) antibodies are nonetheless be found in her serum and that of the neonate (Sontheimer and McCauliffe 1990). The infant may be small for dates and have a scaly annular erythematous and papular rash, mainly on the head and scalp. Any sun or ultraviolet ray exposure will exacerbate the rash. Examination of the skin reveals the changes associated with lupus erythematosus in adults, namely hyperkeratosis, epidermal atrophy, basal cell liquefaction with many colloid bodies, and periappendageal mononuclear cell infiltrates. Remission with or without mild scarring is the usual outcome within a 6-month period (Lee 1993). Other features of lupus may be evident, the commonest being congenital heart block. Indeed, the heart condition may dominate the clinical state, and is probably a cause for an underestimate of the incidence of this condition (Olson and Lindsley 1987).

Graft Versus Host Disease

Graft versus host disease is a systemic condition that can occur when immunocompetent donor lymphocytes circulate in an immunocompromised host. It is most commonly seen following bone marrow transplantation, but it rarely can occur following transplacental transfer of maternal lymphocytes (Alain et al. 1993). The skin manifestations are variable: in the acute phase, there is a rather nonspecific erythematous morbilliform rash. In the chronic phase, the disease is initially lichenoid, and can resemble lichen planus (Saurat and Gluckman 1977) or dermatomyositis (Prussick et al. 1991); later, the skin becomes sclerodermatous (Terasaki et al. 2003). The histology of acute graft versus host disease is characterized by a lichenoid reaction, generally with prominent dyskeratotic cells, associated with an unusually mild lymphocytic infiltrate (Fig. 30.33A); subepidermal separation can occur in more severe forms (see Horn 1994 for grading). Occasional eosinophils may be present, but significant numbers raise the possibility of a lichenoid drug eruption (Canninga-van Dijk

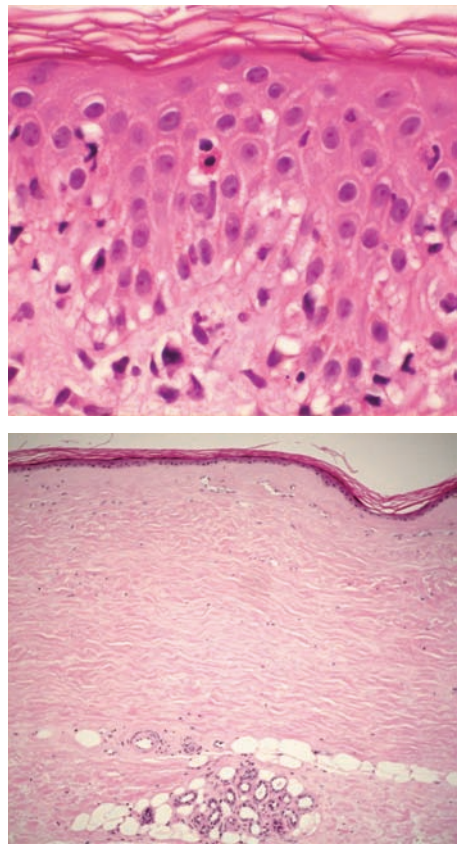


Figure 30.33. Graft versus host disease. (A) Acute phase. Prominent basal cell hydropic degeneration with dyskeratotic cells. (×400) (B) Chronic phase. The epidermis is atrophic, overlying dense sclerosis of the dermis, with entrapped fat. (×200).

et al. 2003). The chronic phase resembles morphea, with dermal sclerosis, often with entrapped fat (Fig. 30.33B). There is usually evidence of overlying lichenoid inflammation, the epidermis being initially acanthotic, but becoming atrophic with time.

Skin Infiltrations and Tumour-Like Lesions

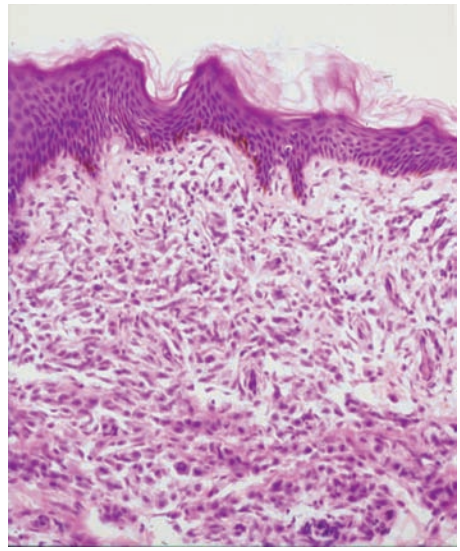
Urticaria Pigmentosa and Diffuse Cutaneous Mastocystosis

Urticaria pigmentosa usually begins in the first year of life as a pigmented maculopapular or nodular rash, most often on the trunk, and occasionally is present at birth. There is mast cell proliferation (hyperplasia) within the superficial dermis and increased melanin pigmentation of basal cells in the overlying epidermis (Fig. 30.34). The skin urticates on stroking, and uninvolved skin often shows dermatographism with a mild generalized increase in dermal mast cells. In most babies, the condition remains confined to the skin and improves gradually with ultimately complete resolution, though sometimes over several years. In a few, the condition remains active for many years, although only very rarely progressing to systemic mastocytosis (Golkar and Bernhard 1997).

A less common variant is solitary mastocystosis (mast cell nevus) in which only one or a few individual lesions are found. Slightly elevated plaques or nodules are evident, often at birth, with histology similar to that of the diffuse condition.

Diffuse cutaneous mastocytosis in infancy is much less common than urticaria pigmentosa and presents as a widespread, diffuse infiltrate of mast cells in the dermis, sometimes with bulla formation. Systemic symptoms of histamine release are present, and there is usually other organ involvement (Travis et al. 1988).

In all examples, a biopsy includes increased numbers of mast cells, often accompanied by small numbers of eosinophils. The mast cells may be difficult to recognize if substantial degranulation has occurred, but this may be minimized by using a local anesthetic without adrenalin and by not injecting this directly into the lesion. Chloroacetate esterase and toluidine blue stains will identify the mast cells. Biopsy material is required to demonstrate systemic spread and, as in the skin, mast cells are aggregated around vessels.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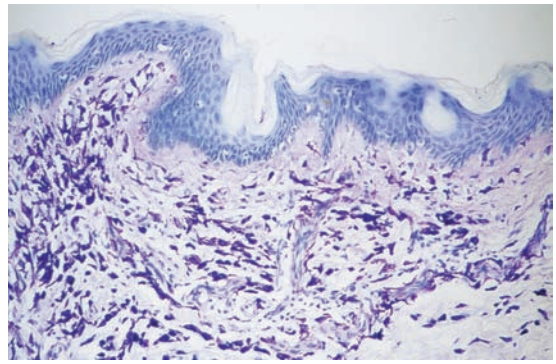

B

Figure 30.34. Urticaria pigmentosa. (A) Mast cells fill the dermis and melanin is prominent in basal cells of the epidermis. (B) Toluidine blue preparations demonstrate metachromatic granules in the cytoplasm of the mast cells and leaking into the dermis.

Subepidermal Calcified Nodules

These nodules, which may be multiple, comprise multiple, variously sized calcific foci in the dermis, and may be referred to as cutaneous calculi (Fig. 30.35). A foreign body reaction can

develop, and the epidermis is often hypertrophic. The condition affects the face principally, and may present either at birth or during childhood, but less commonly in the adult. There is no related metabolic abnormality and the cause is unknown.

Histiocytosis X

A persistent rash may be the initial manifestation in early infancy of the form of histocytosis X known as Letterer–Siwe disease. The rash may be generalized or localized to the perineum, resembling nappy rash, or the scalp, masquerading as seborrheic dermatitis. Onset is usually during the first year of life, and signs of involvement of the bone marrow and reticuloendothelial system follow. Rarely, the disease in infants appears confined to the skin (Wolfson et al. 1981). A dense infiltrate of cells with eosinophilic cytoplasm and convoluted nuclei is found in the dermis (Fig. 30.36A). Scattered mitotic figures are seen, and there is extension of cells into epidermis with occasional ulceration. A few eosinophils are present, but, unlike other forms of histiocytosis X, there are no multinucleate cells, lymphocytes or foamy cells. Electron microscopy of infiltrating cells (Fig. 30.36B), enzyme histochemistry, and positive staining for S100 and CD1a on immunohistochemistry support a Langerhans' cell origin (Bos et al. 1984; Groh et al. 1988; Emile et al. 1995). The prognosis is difficult to assess. Spontaneous remission occurs (Lee et al. 1988) but, with generalized involvement of hemopoietic tissue, a fatal outcome can result (Favara 1991).

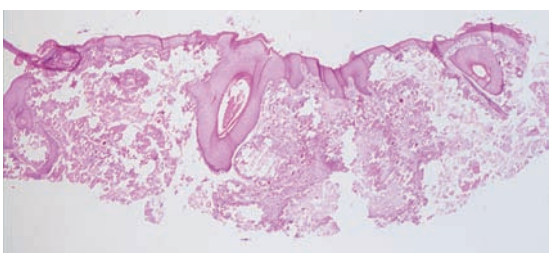
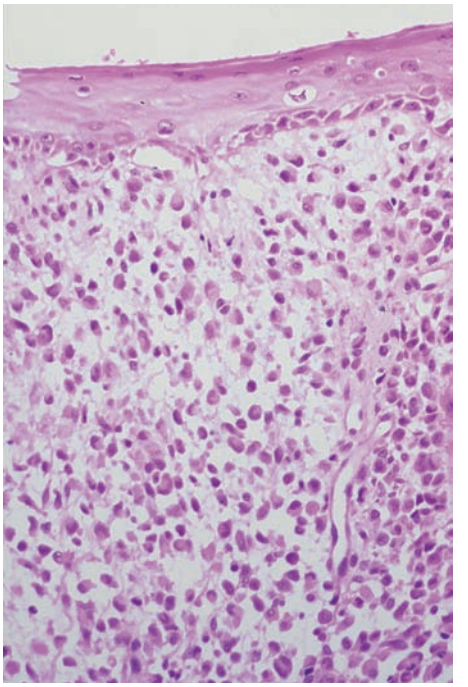
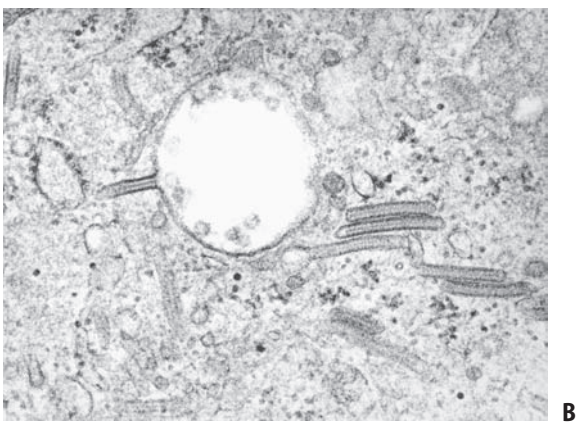


Figure 30.35. Subepidermal calcific nodule. Multiple calcium deposits lie in the dermis. There is no inflammatory reaction.



A



B

Figure 30.36. Histiocytosis X. (A) The dermis is filled with large mononuclear cells, some with reniform nuclei. (B) The ultrastructural characteristic is the scattered Birbeck or tennis racquet granule within the cytoplasm.

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31
The Special Senses

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The Eye

Embryology

A brief description, at the outset, of the anatomy of the eye may help in understanding its embryological development. The eyeball lies suspended within the fat in the orbit that is bounded by four converging orbital bones but unprotected anteriorly where the corneal window lies. The latter is screened by the upper and lower eyelids. The sclera is the tough fibrous connective tissue envelope of the eyeball, and the cornea occupies its anterior aperture. The uveal tract, loaded with blood vessels, lines the inner surface of the sclera. The posterior part of the uveal tract is called the choroid. The choroid extends to close to the corneoscleral junction, and the uveal tract becomes swollen by the fibers of the ciliary muscle to form the ciliary body. Fine fibers converge from the inner surface of the ciliary body to the rim of the lens as its suspensory ligament. The uveal tract continues from the anterior margin of the ciliary body as the iris, whose muscles are disposed as the papillary sphincter and papillary dilator. Aqueous humor oozes from the capillaries of the iris and ciliary muscle and circulates from the posterior chamber (formed by the iris, ciliary body, suspensory ligament, and lens) to the anterior chamber (formed by the cornea and iris), where it disperses into the episcleral veins via an encircling canal of Schlemm. The lens lies in contact with the posterior surface of the iris. The retina, with the rods and cones abutting the choroid surface and the ganglion cells in contact with the vitreous surface, lines the inner surface of the choroid. These ganglion cell fibers course toward the optic disk and leave the eyeball as fibers of the optic nerve.

Thus, at completion of eye development, there are three structural and functional layers: outer—corneoscleral, transparent anteriorly, opaque and fibrous in the remainder; middle—uveal tract (iris, ciliary body, and choroid); and inner—retina. There are two functional compartments: anterior and posterior. The anterior compartment is subdivided into two chambers—the anterior chamber, formed by the cornea, drainage angles, and iris, and the posterior chamber, formed by the iris, ciliary body, suspensory ligament, and lens. The two chambers communicate via the pupillary aperture. The anterior compartment structures are responsible for focusing the image and regulating the intensity of light entering the posterior compartment. The posterior compartment contains the vitreous body and photosensitive retina.

Major stages in ocular development are summarized in Figure 31.1. Some reviews provide a more detailed description of the embryological development of the eye (Strömland et al. 1991; Barishak 2001; Edward and Kaufman 2003).

Optical development begins with bilateral indentations of forebrain neuroectoderm to form the optic sulci, or pits, on day 23 (Fig. 31.1). Each sulcus develops distal bulbous expansions to form the optic vesicles. Invagination of the optic vesicles by the surface epithelium forms the optic cup, while the proximal part of the optic vesicle forms the optic stalk. Simultaneously, invagination of

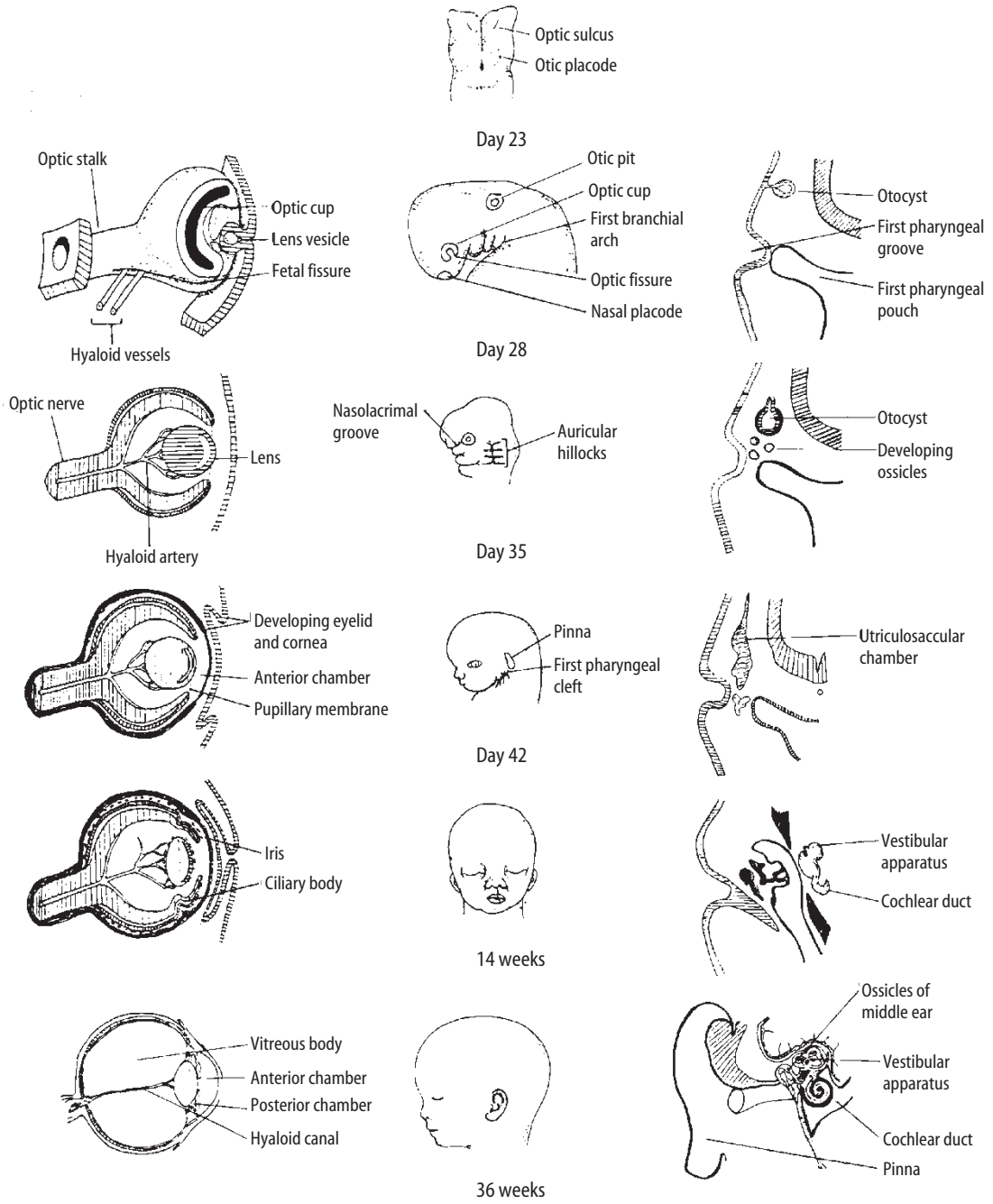

FIGURE 31.1. Diagram summarizing the major stages in the entire development. (Illustration by H. Mellor.)

the optic vesicle commencing inferolaterally and extending into the optic stalk forms a longitudinally orientated cleft, called the optic or choroid fissure, which permits entry of mesodermal tissue by day 28. The outer layer of the optic cup acquires melanin pigment to form the future retinal pigment epithelium, whereas the inner layer differentiates into the neural retina. The primitive

dorsal ophthalmic artery, a large branch of the primitive internal carotid artery, gives rise to the hyaloid artery, which supplies the optic cup from the 4th week of gestation, entering through the posterior aspect of the optic fissure.

At this stage, all major tissue progenitors are present for completion of oculogenesis. The neuroectoderm develops into the optic nerve fibers, the retina, the pupillary sphincter muscles, and the surface epithelium of the iris and ciliary body. The surface ectoderm forms the lens and epithelial components of the cornea, conjunctiva, eyelids, lacrimal glands, and their ducts. The mesoderm differentiates into the hyaloid vessels, extraocular muscles, and fibrovascular tissue of the cornea, iris, ciliary body, choroid, and sclera.

The Lens

The embryonic lens commences development on day 27 from an area of condensed surface ectoderm, the lens placode, which overlies the optic vesicle. The lens placode invaginates, separating from the surface ectoderm during the 6th week to form the lens vesicle. The vesicle expands, developing a central cavity with an anterior wall of cells, which will form the anterior lens epithelium, and a posterior wall, formed by the primary lens fibers, which elongates and obliterates the vesicle cavity. The nuclei of the lens fibers are lost by day 44, increasing their transparency. Later, secondary lens fibers form at the lens equator by division of the anterior epithelium. The lens continues to grow throughout life as cells at the lens equator differentiate into fibers and become displaced toward the center of the lens.

The Retina

As a consequence of the invagination by the optic vesicle to form the optic cup, a double layer of neuroectoderm is formed. The outer layer acquires melanin pigment to form the future retinal pigment epithelium, which develops during the 6th to 12th weeks. The inner layer becomes the multilayered neural retina, becoming trilayered by the 3rd month. Differentiation commences at the posterior pole. The trilayered retina consists of an outer neuroblastic, middle inner neuroblastic, and innermost nonnucleated inner fiber layers. During the 3rd to 6th months there is extensive development of the outer neuroblastic layer, which differentiates into the horizontal and bipolar cells, the rod and cone photoreceptors. By the 22nd week the retina is composed of 10 layers similar in structure to that in the adult. The innermost retinal layer is the optic nerve fiber layer formed by ganglion cell axons that merge to form the optic nerve and exit the eye at the optic disk.

Further retinal and optic nerve maturation occurs during the first year of life. The retina is vascularized from two sources: the outer nucleated photoreceptor-containing lamina receives nutrients indirectly from the choroidal capillaries by active transport across the retinal pigment epithelium; the inner laminae are supplied by the central retinal vessels (which originate from the hyaloid vessels) and vascularize the inner retina in a radial pattern. The peripheral portion of the temporal retina is the last area to become vascularized and reaches completion around the 8th month of gestation. This is of clinical significance in the pathogenesis of retinopathy of prematurity.

The Choroid, Sclera, Ciliary Body, Iris, Cornea, and Anterior and Posterior Chambers

The choroid develops from the mesoderm surrounding the optic vesicle. The sclera is formed by condensation of mesoderm surrounding the choroidal mesoderm.

The iris dilator and sphincter muscles develop from neuroectoderm at the margin of the optic cup around the 6th to 8th month of gestation. The mesoderm situated on the margin of the optic cup opening forms the smooth muscle and connective tissue of the ciliary body and suspensory ligaments of the lens. The iris stroma is derived from the neural crest. The cornea is derived from three waves of neural crest cells with most of the development between the 6th week and 5th month of gestation.

The chamber angle starts developing between the 12th and 14th week. The anterior chamber angle is well formed by the 8th month. The posterior chamber develops from a cleft-shaped split between the posterior surface of the pupillary membrane and the anterior capsular surface of the lens. The chambers gradually fill with aqueous humour and communication is

established between the two chambers via the pupillary aperture. Aqueous formation and drainage are evident by 18th week.

The Vitreous Body

The vitreous body develops in three stages. During the 5th week the primary vitreous forms from a vascular matrix of fibrils originating from the retinal neuroectoderm and lens ectoderm. The primary vitreous atrophies and is replaced by the secondary vitreous during the 9th week from a retina-derived gelatinous acellular matrix, which is rich in proteoglycans and collagen fibrils. The tertiary vitreous develops in the 4th month of gestation as anterior condensations of collagen fibrils that form the zonular fibers that attach the lens capsule to the ciliary body processes. Persistence of the primary vitreous causes visual impairment.

The Hyaloid Vessels

The primitive dorsal ophthalmic artery, a large branch of the primitive internal carotid artery, gives rise to the hyaloid artery, which supplies the optic cup from the 4th week of gestation. When the development of the lens vesicle and the primary vitreous body is achieved, around day 64, the vessels degenerate, leaving a vestigial remnant, the acellular canal of Cloquet, which may be visible at birth. A branch of the hyaloid artery, the tunica vasculosa lentis, supplies the lens during the 12th to 28th weeks. Between the 28th and 38th weeks this degenerates, but embryological remnants of the tunica vasculosa lentis, known as Mittendorf's dots, may be detected during funduscopic examination of the eye.

The Extraocular Muscles

The extraocular muscles are derived from paraxial mesoderm and become innervated by the third, fourth, and sixth cranial nerves.

The Eyelids

The eyelids develop peripherally to the optic vesicle as folds of surface ectoderm during the 7th week and grow centripetally to fuse by the 9th week, enclosing a cleft-shaped space, the conjunctival sac, anterior to the cornea. At about the 5th month of fetal life, the eyelids begin to separate, starting nasally and extending laterally, to be normally completely separated by the end of the 7th month. Eye lash and lid sebaceous glands develop from the surface epithelium.

The Accessory Ocular Glands

The lacrimal gland is formed from epithelial buds arising from the epithelial layer of the conjunctiva and continues to grow during fetal life. The nasolacrimal duct begins as a solid root of ectodermal cells that become progressively canalized superiorly from the 3rd month of gestation and is normally completed by about the 6th month.

Genetic Regulation of Ocular Development

The gene that appears to be critical to ocular development is the *Pax6* gene (van Heyningen and Williamson 2002; Hanson 2003; Treisman 2004). The transcription factor, Pax6, is present in almost all eye structures and is thought to have primacy. The *Pax6* gene is located on chromosome 11p13. Mutations of *Pax6* lead to aniridia, cataract formation, Peter's anomaly, and optic-nerve malformations (Azuma et al. 2003), while a complete loss of *Pax6* function leads to failure of eye formation. There are numerous upstream or downstream interactions with *Pax6* that add to the complexity of the gene interactions (van Heyningen and Williamson 2002). For example, *Sox2*, a downstream target of *Pax6*, may play a crucial role in eye specification, as the loss of *Sox2* can result in anophthalmia or microphthalmia (Ragge et al. 2005), a more severe phenotype than aniridia caused by the loss of one copy of *Pax6* (Treisman 2004).

Genetic heterogeneity in ocular anomalies can be illustrated by Axenfeld-Rieger malformations, which can be syndromic or nonsyndromic. Genes at different loci can cause the malformation, both in its syndromic or nonsyndromic forms. However, while mutations in the homeobox transcription factor PITX2, which maps to 4q25, show strong genotype/phenotype correlations, those due to a forkhead transcription factor FOXC1, which maps to 6p25, show weak genotype/phenotype correlations (Saleem and Walter 2002).

The *RAX/RX/Rx* gene maps to chromosome 18. *Rx* mutants have shown abnormalities, such as failure of differentiation of the optic vesicle, in experimental animals, and mutations in the human *RAX* gene have confirmed a role for this gene in a patient with anophthalmia and sclerocornea defects (Voronina et al. 2004).

Postnatal Development of the Eye

The eye continues to grow after birth until adolescence but the changes are small. The cornea, 2 to 3 µm at birth, reaches a final thickness of 8 to 16 µm in adult life. The fovea matures during the first 4 months, providing maximum exposure of the photoreceptors to incident light. Term infants generally respond poorly to visual targets, but the presence of vision can be demonstrated by pupil responses or aversive behavior to bright lights. The size of the anteriorly located focusing apparatus increases more rapidly than the remaining eye structures during the first year. The intensity of pigmentation of the iris and retina also increases. Myelination of the optic nerve fibers is incomplete until the 3rd month. Color vision is evident by 2 months and matures to adult levels by 6 months; accommodation develops by 4 months, and three-dimensional vision is evident by 6 months of age.

The structural and functional changes after birth are of particular significance when ocular assessment of preterm infants is attempted, since normal intrauterine development will be incomplete. A small number of term infants show functional delay of visual maturation, in isolation from other neurological abnormality.

Abnormalities of Periocular Tissues

As a bridge between the face and the cranium, the orbits represent an important resource for clinical dysmorphology. Standard measurements of ocular landmarks include the inner canthal distance (ICD), the outer canthal distance (OCD), the interpupillary distance (IPD), and the horizontal palpebral length. The orientation of the palpebral fissures can be described also (Levin 2003; Dollfus and Verloes 2004). Other features that are sought include characteristics of the eyelids and positioning of the globes.

The list of syndromes associated with abnormal ocular biometrics is lengthy, and it is beyond the scope of this chapter to encompass all of them. Examples of these associations include holoprosencephaly with hypotelorism; Apert syndrome, Crouzon syndrome, and Coffin-Lowry syndrome with hypertelorism; trisomy 21 with upward slanting palpebral fissures; and Coffin-Lowry syndrome and Noonan syndrome with downward slanting palpebral fissures. Many of these features of abnormal ocular biometrics are illustrated in *Smith's Recognizable Patterns of Human Malformation* (Jones 1997).

Developmental Abnormalities of Oculogenesis

The temporal relationship of ocular development and some malformations is illustrated schematically in Table 31.1. Estimates of congenital ocular malformations range from 60 in 100,000 to 75 in 100,000 births (EUROCAT Working Group 1995; Stoll et al. 1992). However, it must be recognized that some ocular anomalies may not be readily recognized at, or shortly after, birth (Rahi and Dezateaux 2001), can be subject to underascertainment (Campbell et al. 2002), or complicated by definitions, particularly the terms *anophthalmia/microphthalmia* and *cyclopia/synophthalmia*, which are used different ways by different authors and by different disciplines.

Anomalies in other organ systems are present in about 50% of infants with ocular malformations (Stoll et al. 1992; Bermejo and Martinez-Frias 1998). Microcephaly, hydrocephaly, cleft lip, and palate and other facial dysmorphisms were the most common. The eye anomaly was part of a recognized syndrome complex in about 29% of cases (Table 31.2) (Bermejo and Martinez-Frias 1998). Sixty percent of syndromes in their survey had abnormal chromosomes (Table 31.3). Details of the individual ocular abnormalities present in common syndrome complexes are found in Jones (1997). Sound data about ocular teratogens accumulate slowly, and little is known about the effect of many putative environmental agents on the developing eye.

There is better information about the effect of various maternal conditions such as diabetes and,

Table 31.1. Chronological development of the eye and developmental anomalies

Ge stational age	Developmental milestone	Developmental anomaly
22 days	Optic primordial appears	Anophthalmia; cyclopia; cystic eye; nonattachment of retina
2nd month	Hyaloid artery fills embryonic fissure	
	Closure of embryonic fissure begins	Coloboma
	Lid folds appear	
	Neural crest cells migrate centrally; corneal stroma follows	
	Choroidal vasculature starts to develop	
	Axons from ganglion cells migrate to optic nerve	
3rd month	Sclera condenses	
	Lid folds meet and fuse	
4th month	Retinal vessels grow into nerve fiber layer near optic disk	
	Schlemm's canal appears	
	Glands and cilia develop in lids	
5th month	Photoreceptors develop inner segments	
	Lids begin to separate	
6th month	Dilator muscle of iris forms	
7th month	Central fovea thins	
	Fibrous lamina cribrosa forms	
	Choroidal melanocytes produce pigment	
8th month	Iris sphincter develops	
	Chamber angle completes formation	Anterior chamber cleavage syndromes
	Hyaloid vessels regress	
	Retinal vessels reach periphery	Retinopathy of prematurity
	Myelination of optic nerve is complete to lamina cribrosa	
	Pupillary membrane disappears	Pupillary membrane

Table 31.2. Syndromes where disordered oculogenesis is usual

Disease	Inheritance	Ocular changes
Norrie's disease	XLR	Shallow anterior chamber, cataract, atrophic iris, ectropion uvea, retrolental mesodermal mass, disorganized retina, atrophic optic nerve.
GM_1 gangliosidosis	AR	Macula–cherry red spot at birth; deposits of glycolipid in retina
Walker-Warburg syndrome	AR	Retinal dysplasia, anterior chamber malformation, microphthalmia, retrolental mass, coloboma
Cerebro-oculofacioskeletal (COFS) syndrome	AR	Blepharophimosis, microphthalmia, cataracts
Oculoauricularverte bral spectrum	Usually sporadic	Epibulbar dermoid, eyelid coloboma
Oculodentodigital syndrome	AD	Microphthalmos, microcornea, cataract
Aniridia-Wilms' tumour assoc. (WAGR)	Sporadic/familial (del 11p13)	Aniridia, congenital cataracts, blindness, nystagmus, ptosis
CHARGE association	Uncertain	Coloboma (iris, retina/clinoid, optic nerve), nystagmus

AR, autosomal recessive; AD, autosomal dominant; CHARGE, coloboma, heart disease, atresia choanae, retarded growth and development, genital hypoplasia, and ear anomalies; XLR, X linked recessive.

Table 31.3. Some chromosomal abnormalities that affect ocular development

Disorder	Ocular abnormalities
Trisomy 13	Synophthalmia, microphthalmia, corneal opacity, coloboma, anterior chamber malformations, cataract, PHPV, retinal dysplasia, retrolental mesodermal mass
Trisomy 18	Exophthalmos, microphthalmia, corneal opacity, cataract, anterior segment malformation, iris and ciliary process abnormalities, coloboma, retinal folds, retinal dysplasia
Trisomy 21	Cataract, Brushfield's spots on iris, ectropion uvea, optic nerve hypoplasia
Triploidy syndrome	Microphthalmia, coloboma, synophthalmia
Deletion 13q	Retinoblastoma (bilateral) keratoconus, microphthalmia, coloboma, retinal dysplasia
Partial deletion 11p	Corneal opacity, aniridia, cataract, macular hypoplasia, optic nerve hypoplasia, coloboma
Deletion 4p	Microphthalmia, coloboma
Deletion 5p	Optic atrophy

PHPV, persistent hyperplastic primary vitreous.

Table 31.4. Ocular teratogenic effects (Strömland et al. 1991)

Teratogen	Related ocular anomalies
Ionizing radiation	Microphthalmia, cataracts, retinal pigmentation disorders
Hyperthermia	Microphthalmia
Rubella infection	Microphthalmia, microcornea, anterior angle closure, iris anomalies, chorioretinitis, pigmentary retinopathy, glaucoma, strabismus, nystagmus
Cytomegalovirus infection	Microphthalmia, optic nerve hypoplasia, coloboma, optic atrophy, cataracts, anophthalmia, Peter's anomaly, chorioretinitis
Varicella-zoster virus infection	Chorioretinitis, microphthalmia, atrophy/hypoplasia of optic disc, cataract, Horner's syndrome
Toxoplasmosis	Chorioretinitis
Syphilis	Intestinal keratitis opacity. Less commonly chorioretinitis, glaucoma, cataract, uvertis
Maternal diabetes	Optic nerve hypoplasia, coloboma, septo-optic dysplasia
Anticonvulsants	Epicanthic folds, telecanthus, strabismus, ptosis, optic nerve hypoplasia; infrequent anomalies include microphthalmia, coloboma, retinal dysplasia
Retinoic acid	Small palpebral fissures, microphthalmia, hypertelorism
Warfarin anticoagulants	Cataract, optic atrophy, optic nerve hypoplasia, small eyelids, hypertelorism
Thalidomide	Microphthalmia, anophthalmia, lens anomalies, coloboma, motility disturbance—several, buphthalmos
Alcohol	Microphthalmia, short palpebral fissures, telecanthus, strabismus, ptosis, myopia, Peter's anomaly, optic disk hypoplasia, optic nerve hypoplasia, glaucoma, microcornea, cataract, retinal vascular tortuosity, persistent hyperplastic primary vitreous

particularly, infection early in pregnancy. Ocular abnormalities can result when certain drugs are administered in the first trimester (Table 31.4) (Strömland et al. 1991). All produce a recognized spectrum of anomalies and are avoidable substances when pregnancy is planned.

An increase in maternal drug ingestion in pregnancy is described in babies with ocular malformation when compared with controls (Stoll et al. 1992). This study also noted an association with maternal epilepsy and paternal occupational hazards. Adverse environmental factors (predominantly congenital infection) were found in 10% of a series from Spain (Bermejo and Martinez-Frias 1998).

Abnormalities of the Globe

Anophthalmia/Microphthalmia

Anophthalmia is the apparent absence of the globe in an orbit that otherwise contains normal adnexal structures. Anophthalmia can be unilateral or bilateral. When unilateral, there is usually contralateral microphthalmia. True anophthalmia, resulting from a failure of optic vesicle development or its disruption early in development, is extremely rare. "Clinical anophthalmia," referring to an inapparent microphthalmic globe, is probably a better description of the commoner finding (Fig. 31.2) and vestigial structures may be found within the orbit on microscopic examination (Warburg et al. 1997).

Microphthalmia is defined as an eye measuring less than 15 mm in maximum diameter (Fig. 31.3). It is described as part of a large number of syndromes (Warburg 1991).

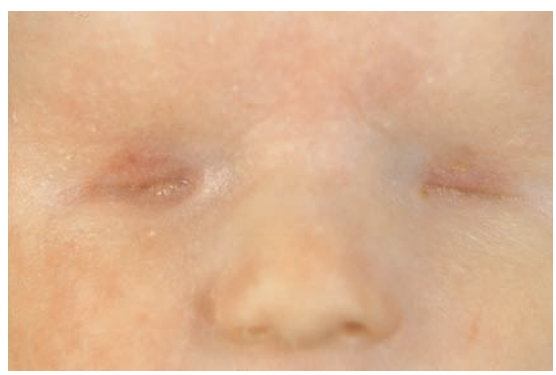

Figure 31.2. Bilateral anophthalmia in a neonate. Palpebral fissures are small and set back into small, empty orbits.

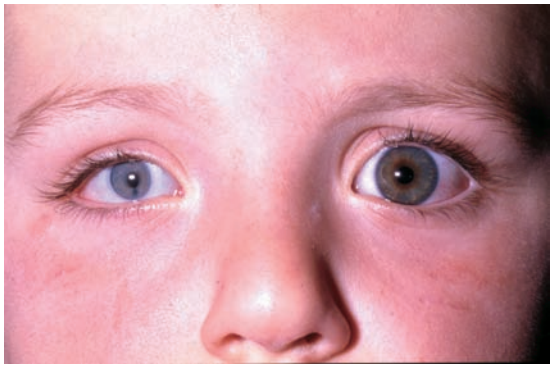

Figure 31.3. Microphthalmia of the right eye with typical iris coloboma in inferior aspect. (Courtesy of Doug Coster, Flinders Medical Centre, Bedford Park, Australia.)

An autosomal recessive form of isolated anophthalmia has been described (Oliveira da Silva and Santana de Sousa 1981). Mutations in the *SOX2* gene, located at 3q, has been shown to cause bilateral anophthalmia (Fantes et al. 2003). Microphthalmia has many causes, coloboma being the most frequent (Warburg et al. 1997). It was found in six of 11 children with mental retardation exposed to hyperthermia in utero at between 4 and 7 weeks' gestation (Pleet et al. 1981). Microphthalmia/anophthalmia are associated with rubella, cytomegalovirus, influenza, and Parvovirus infections (Busby et al. 2005).

No clustering to suggest an environmental factor in the etiology of anophthalmia and microphthalmia has been found in Britain, Sweden, or Canada (Dolk et al. 1998; Kallen and Tornqvist 2005; Lowry et al. 2005). A review of the European Concerted Action on Congenital Anomalies and Twins (EUROCAT) data set from the Netherlands and a review of 13 studies in the literature did not support an association between microphthalmia/anophthalmia and maternal carbamazepine medication (Kroes et al. 2002).

Nanophthalmia

Nanophthalmia is an uncommon form of bilateral microphthalmia that is caused by an autosomal dominant disorder. There is a proportional decrease in size of all the internal architectural structures associated with an abnormally thick sclera.

Cyclopia/Synophthalmia

Fusion of the optic vesicles during the first month of gestation results in fusion of the eyeballs, which can vary from a single globe within a single orbit (cyclopia) to "fused" or duplication of globes within a single orbit (synophthalmia) (Fig. 31.4). True cyclopia is very rare while synophthalmia is encountered more commonly. Both are seen more frequently in females (Cohen 1989a) and twins. The anterior part of the eye(s) are often well formed and the associated optic nerve is also abnormal (Kakita et al. 1997). Diplophthalmia within a single lateralized orbit with a normal contralateral eye has been described; associated anomalies suggest that this represents a disruption later in embryonic development (Hausmann et al. 1992).

Both cyclopia and synophthalmia are accompanied by severe facial dysmorphism and gross central nervous system (CNS) anomalies. The latter are usually at the severe end of the holoprosencephaly spectrum, most frequently alobar holoprosencephaly but occasionally with aprosencephaly (see Chapter 26) (Lurie et al. 1992). The intervening skull base has related and predictable anomalies (Kjaer et al. 1991). Synophthalmia is also described with defects of neural tube closure, anencephaly (holocrania) and rachischisis (Lemire et al.

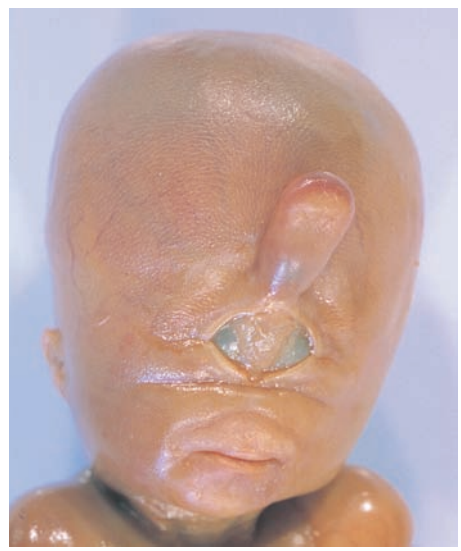

Figure 31.4. Synophthalmia. Fused globes are present within a diamond-shaped central palpebral fissure. There is a supraorbital proboscis.

1981), occipital encephalocele (Cohen et al. 1972), and iniencephaly (Ramakrishnan et al. 1991).

Chromosome anomalies are frequent in the cyclopic fetus, which is not surprising given the frequent association with holoprosencephaly (Cohen 1989a,b). Cyclopia is also seen in some autosomal recessive holoprosencephaly cases and in certain syndromes which include holoprosencephaly, such as agnathia (hypognathia)/holoprosencephaly syndromes (Cohen 1989a).

Cyclopia is described following congenital cytomegalovirus infection and maternal ingestion of high-dose salicylates in the first trimester (Benawra et al. 1980; Agapitos et al. 1986).

Cryptophthalmos

In cryptophthalmos (covered eye), there is failure of eyelid separation and absence of eyelashes and palpebral fissure. It can be unilateral or bilateral, and it frequently coexists with other ocular anomalies. Isolated cryptophthalmos may be familial or sporadic (Thomas et al. 1986). A dominant syndrome is also recorded (Saal et al. 1992). Cryptophthalmos is a major criterion of the cryptophthalmos/syndactyly (Fraser) syndrome (Fig. 31.5), a recessively inherited disorder, where it is described in 88% of recorded cases (Slavotinek and Tifft 2002). At the severe end of the spectrum are malformations of the larynx and genitourinary tract, craniofacial dysmorphism, orofacial clefting, mental retardation, and musculoskeletal anomalies (Slavotinek and Tifft 2002).

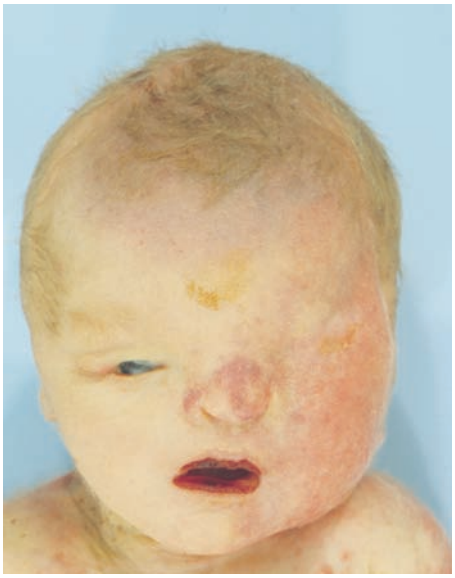

Figure 31.5. Unilateral cryptophthalmos in Fraser syndrome. The underlying eye was developmentally abnormal.

Globe Enlargement

Eye enlargement (macrophthalmia) may occur with buphthalmos, where there is enlargement of the entire eye from primary congenital glaucoma. A new syndrome of microcornea, coloboma, and macrophthalmia that has an autosomal dominant pattern of inheritance has been described (Toker et al. 2003).

Colobomas

The majority, referred to as "typical" colobomas, result from the persistence of a cleft-shaped fissure situated on the inferonasal aspect of the eye, resulting from incomplete fusion of the fetal fissure during the 6th week of development. The term *atypical coloboma* refers to a cleft-shaped defect occurring in any other eye quadrant. Vestiges of the anterior hyaloid vessels and pupillary membrane often occur in association with these defects and it may be their persistence that impairs normal ocular development in the quadrant affected. The defective closure of the fissure can affect the eyelid (Fig. 31.6), iris (Fig. 31.7), ciliary body, retina (Fig. 31.8), choroid, or optic nerve.

Coloboma is most often seen as a sporadic defect, but it is also seen in a large number of syndromes (Warburg 1991). Associated anomalies may be restricted to the eye (Pallotta et al. 1998), include facial structures and brain or involve many other systems. *PAX2* mutations are seen in the renal-coloboma syndrome (Schimmenti et al. 2003), while *PAX6* mutations have been described in sporadic atypical coloboma (Vincent et al. 2004); *PAX2* and *PAX6* downregulate the expression of the other (Azuma et al. 2003).

Coloboma is also seen following exposure to thalidomide or D-lysergic acid diethylamide (LSD) in the embryonic period.

Developmental Abnormalities of the Cornea

Developmental anomalies of the cornea include the following:

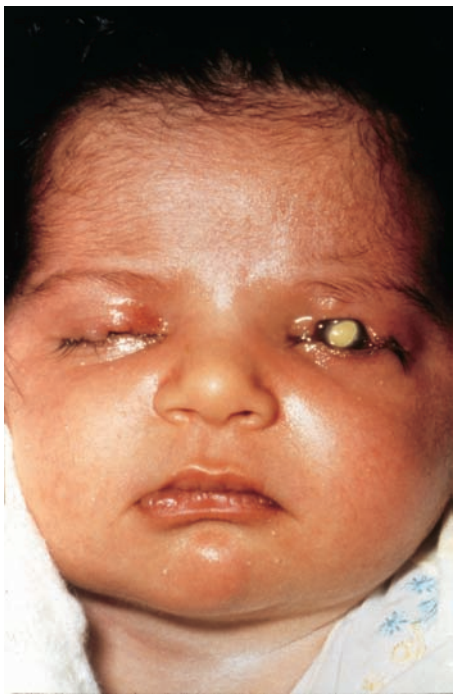

Figure 31.6. Eyelid coloboma. [From Taylor D (ed) (1992) Slide Atlas of Pediatric Ophthalmology, Blackwell Scientific Publications, Oxford, with permission.]

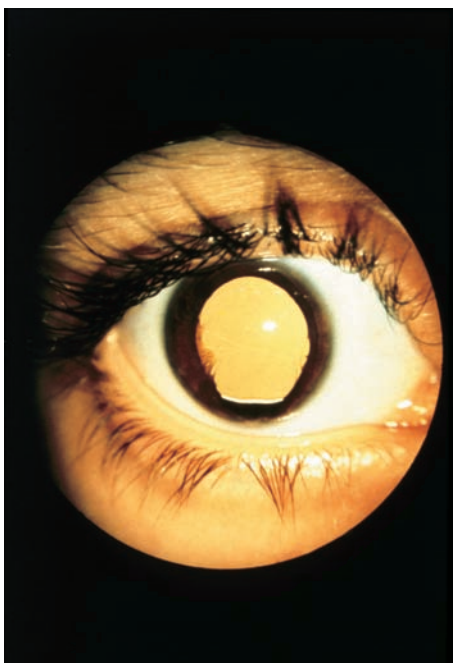

Figure 31.7. Iris coloboma. [From Taylor D (ed) (1992) Slide Atlas of Pediatric Ophthalmology, Blackwell Scientific Publications, Oxford, with permission.]

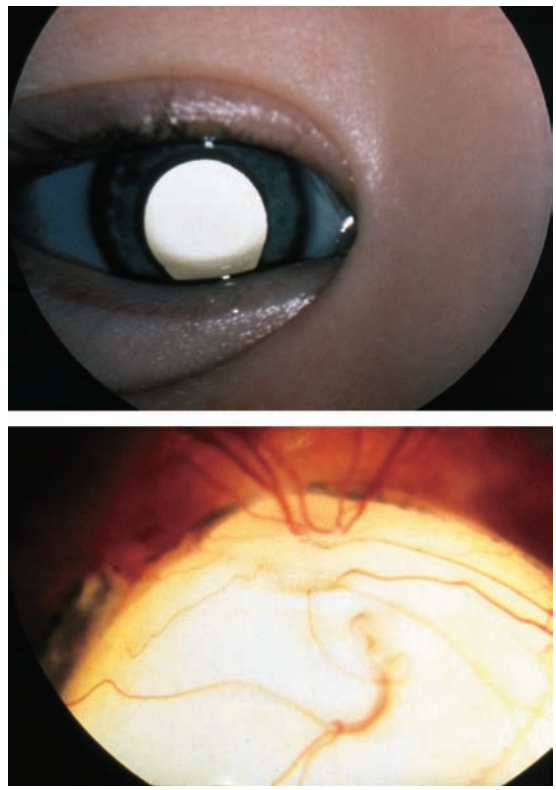

Figure 31.8. Retinal coloboma. [From Taylor D (ed) (1992) Slide Atlas of Pediatric Ophthalmology, Blackwell Scientific Publications, Oxford, with permission.]

- *Microcornea:* a cornea measuring <11 mm diameter in an otherwise normal eye.
- *Megalocornea:* a cornea measuring >13 mm diameter. It is usually bilateral and affects males more frequently than females. Megalocornea may be inherited as an autosomal dominant, autosomal recessive, or X-linked recessive condition. Eyes with megalocornea usually have myopia, and sometimes there is hypoplasia of the iris stroma, cataract, ectopia lentis, and a persistent papillary membrane. Marfan syndrome, craniosynostosis, lamellar ichthyosis, and mental retardation may be associated. In the fifth to sixth week of embryonic life the growing rim of the optic cup slows and may not bend axially such that the ciliary ring and anterior segment of the globe become abnormally large.
- *Sclerocornea:* opacification of the peripheral cornea that results from a defect in alignment and caliber of the corneal fibrils.

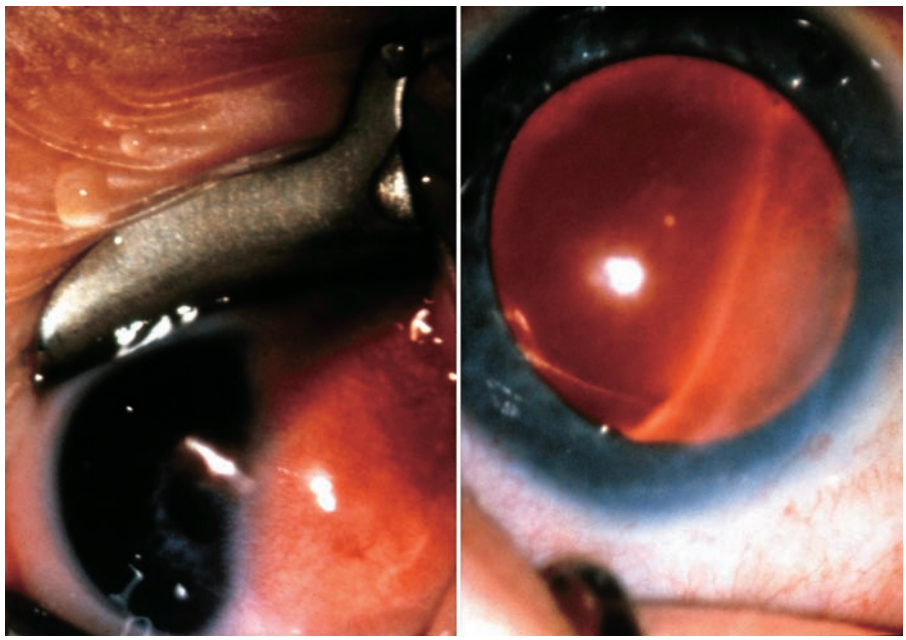

Figure 31.9. Congenital limbal/corneal dermoid. [From Taylor D (ed) (1992) Slide Atlas of Pediatric Ophthalmology, Blackwell Scientific Publications, Oxford, with permission.]

- *Dermoids:* These may be central or limbal in location (Fig. 31.9). They are hamartomas arising due to arrested migration of ectoderm at the corneoscleral junction (limbus), most commonly on the temporal aspect. Most corneal dermoids are solitary, but they can be part of Goldenhar's syndrome and may be associated with ocular anomalies such as microphthalmos.
- *Congenital hereditary corneal dystrophy:* This is a bilateral noninflammatory disorder of the cornea that has autosomal dominant and recessive and, rarely, X-linked recessive inheritance patterns. It is classified arbitrarily according to the layer involved—anterior, stroma, or endothelial—but many conditions affect more than one layer. Corneal clouding is present from birth due to edema of the basal layer of the epithelium, subepithelial fibrosis, reduction of the thickness of Bowman's membrane, or central attenuation of the endothelial cell density (Kirkness et al. 1987).

Anterior Chamber Dysgenesis

Anterior chamber dysgenesis (anterior chamber cleavage syndrome, anterior segment dysgenesis, mesenchymal dysgenesis) describes a group of anomalies where there is incomplete migration or differentiation of the mesenchymal cells that contribute to the development of the cornea, iris, and lens, resulting in distortion of anterior chamber architecture and the filtration angle apparatus. It predisposes to the development of glaucoma. While many of the changes leading to normal iridocorneal angle structure occur in the third trimester, insults during the 5th to 7th weeks of gestation can affect subsequent development.

Anterior chamber dysgenesis is traditionally classified based on the clinical phenotypes, but it is now clear that the disorders are a spectrum of expressivity of disease phenotypes (Gould and John 2002). These disorders include Axenfield's anomaly, Peter's anomaly, Rieger's anomaly, aniridia, iridogoniodysgenesis and posterior embryotoxon. Congenital glaucoma is usual with Peter's anomaly and Rieger's anomaly.

Anterior segment anomalies are seen in several chromosome anomaly syndromes, particularly trisomies 13, 18, and 21 and partial deletion of chromosomes 3, 10, 11, and 18 and as manifestations of the fetal alcohol syndrome (Miller et al. 1984). Several genes, including those encoding

PITX2 and FOXC1 (Walter 2003), are known to cause anterior chamber dysgenesis, but the complexity and spectrum of the disorders is underlined by the heterogeneity of genes involved and the lack of a specific phenotype associated with specific genetic mutation (Gould and John 2002; Wiggs 2005).

Congenital Glaucoma

In primary congenital glaucoma raised intraocular pressure is present at birth or develops in the neonatal period. The anterior segment of the eye is abnormal, with incomplete development of the trabecular meshwork and outflow tracks leading to impairment of the flow of aqueous humour. Both eyes are usually affected; presenting features include photophobia, lacrimation, buphthalmos (globe and corneal enlargement), and eye rubbing. Congenital glaucoma is inherited as an autosomal recessive trait and is prevalent in countries where consanguinity is common (Wiggs 2005). Defects in the gene coding for CYP1B1, a protein of the cytochrome P-450 family, have been described (Stoilov et al. 1998).

Developmental glaucoma defines glaucoma resulting from abnormal development of the anterior segment of the eye (Libby et al. 2005). These include Axenfeld-Rieger syndrome and the anterior segment dysgenesis syndrome. These disorders are all inherited as autosomal dominant traits, and the genes responsible have been localized to various chromosomes (Wiggs 2005), but the variable expressivity and incomplete penetrance point to a multifactorial etiology (Gould and John 2002). The variable expressivity may be due to modifier genes or dosage effects.

Developmental Abnormalities of the Iris

Aniridia

Aniridia refers to hypoplasia rather than aplasia of the iris and may occur sporadically or as an autosomal dominant disorder. Aniridia linked to mutation of the *PAX6* gene at 11p13 is associated with increased risk of nephroblastoma (Hanson 2003). Other anomalies such as persistent pupillary membrane, optic nerve hypoplasia, foveal hypoplasia, and cataract are seen, and it predisposes to the development of secondary congenital glaucoma. A spectrum of iris anomalies is seen in some families (Hamming et al. 1986). Mis-sense mutation of *PAX6* has been demonstrated in some atypical cases (Hanson 2003).

Iris Coloboma

See Colobomas, above.

Developmental Abnormalities of the Lens

Abnormalities of the crystalline lens that may present at birth or in the neonatal period include the following:

- Congenital absence of the lens (aphakia): This rare condition is due to failure of lens vesicle formation (primary) or degeneration after the vesicle develops (secondary). It is associated with severe microphthalmos, coloboma, and anterior segment aplasia. Primary aphakia is occasionally seen in syndromes where gross ocular anomalies are frequent (Johnson and Cheng 1997). Secondary aphakia can occur in congenital infections.
- Abnormalities of lens shape: In lenticonus there is a conically shaped anterior or posterior surface of the lens (Fig. 31.10). Anterior lenticonus is associated with cataract formation and Alport's syndrome. In lentiglobus the lens is hemispherically shaped.
- Abnormalities of lens size: Microphakia, small lens, is associated with Lowe's syndrome. Microspherophakia, small spherical lens, is associated with hyperlysinemia and Marfan and Weill–Marchesani syndromes.
- Abnormalities of lens position: Ectopia lentis results from zonular rupture, causing lens dislocation or subluxation. It causes secondary congenital glaucoma and is associated with hyperlysinemia, sulfite oxidase deficiency, homocystinuria, buphthalmos, and Marfan, Weill-Marchesani, and Ehlers-Danlos syndromes.
- Congenital cataracts: The lens is opacified (Fig. 31.11); it is the leading cause of visual disability in children. In developed countries congenital cataracts occur with a frequency of 30 in 100,000 births, with rates being higher in developing

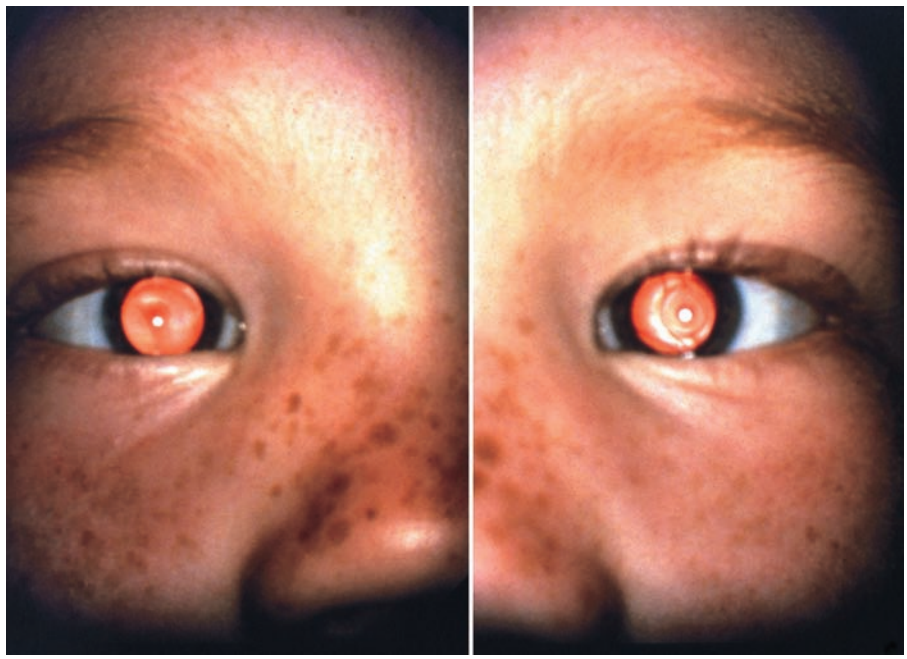

Figure 31.10. Posterior lenticonus. [From Taylor D (ed) (1992) Slide Atlas of Pediatric Ophthalmology, Blackwell Scientific Publications, Oxford, with permission.]

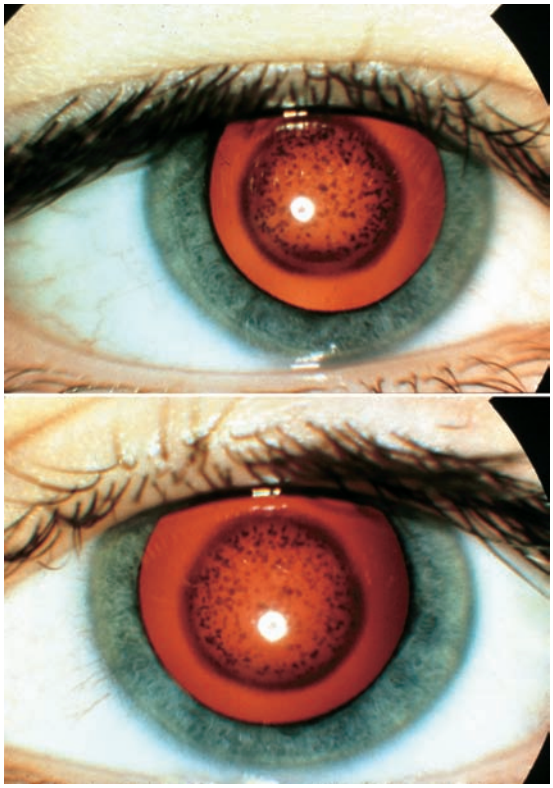

Figure 31.11. Bilateral lamellar congenital cataracts. [From Taylor D (ed) (1992) Slide Atlas of Pediatric Ophthalmology, Blackwell Scientific Publications, Oxford, with permission.]

countries due to consanguinity and rubella infections (Graw 2004). Up to a quarter of cases may have a hereditary component. In nonconsanguineous populations, inheritance of nonsyndromic cataract is most commonly autosomal dominant, but X-linked and autosomal recessive forms are also seen. Even in populations where consanguinity is prevalent, autosomal dominant inheritance is reported more often than autosomal recessive disease. Fifteen specific genes associated with isolated inherited cataract have so far been identified, and there is genotype-phenotype heterogeneity (Reddy et al. 2004). Congenital cataract can also occur in association with other intraocular anomalies such as aniridia, persistent hyperplastic primary vitreous, and vitreoretinal disease. Bilateral cataracts are seen in many syndromes, including genetic metabolic disorders and following intrauterine infection, such as rubella, although such insults generally give rise to systemic malformations in addition to the cataract.

Persistence Hyperplastic Primary Vitreous

The primary vitreous, a gelatinous matrix derived from mesoderm and that includes the hyaloid vessels, is normally resorbed during the second trimester. However, mesodermal remnants may persist either anteriorly (more commonly) or posteriorly, within the posterior compartment. The presence of such remnants has been termed persistent hyperplastic primary vitreous (PHPV) (Reese 1955) and can be defined as an idiopathic persistence and proliferation of the normally transient primary vitreous vasculature and matrix, leading to formation of a retrolental mass with subsequent visual impairment. The term *persistent fetal vasculature* has been suggested as a more appropriate designation.[11] Histologically, there is a retrolental mass containing hyaloid vessel remnants.

The condition is usually sporadic and unilateral, presenting in the neonatal period as microphthalmia with a white pupillary reflex (leukocoria) and a funnel-shaped retrolental mass with its apex attached to the optic disk (Pollard 1997). Bilateral PHPV has been described in children with neurological abnormalities (Marshman et al. 1999) and in a family with osteoporosis–pseudoglioma syndrome (Steichen-Gersdorf et al. 1997).

Other ocular abnormalities that coexist with PHPV include microcornea, shallow anterior chamber, ectropion uvea, atypical iris coloboma, pupillary membrane remnants, lens coloboma, cataract, and retinal detachment.

Localized persistence of the primary vitreous remnant on the posterior aspect of the lens capsule is recognized clinically as Mittendorf's dots and hyperplastic posterior vascular remnants, which may or may not be accompanied by glial proliferation on the optic disk, as Bergmeister's papilla.

Developmental Abnormalities of the Retina

A variety of developmental disorders can affect retinal morphology and function, including the following:

- *Congenital abnormalities of retinal photoreceptors:* The most notable is congenital achromatopsia (monochromatism). Two variants are recognized:
- *Complete rod monochromatism* (autosomal recessive), when there is complete absence of the cone photoreceptor development.
- *Cone monochromatism*, when the rod photoreceptor cells are morphologically and functionally normal; only one subtype of functionally active cone photoreceptor cell develops.
- *Leber's congenital amaurosis:* This is an inherited retinal dystrophy that has an autosomal-recessive pattern of inheritance in most cases. It accounts for 10% to 15% of congenital blindness. Some children only exhibit blindness of retinal origin, but others may have multisystem disorders that may include renal, cardiac, skeletal, and CNS anomalies (Fazzi et al. 2003). Other ocular defects may be present including keratoconus (central thinning of the cornea), cataract, and variable degrees of retinal pigmentation. The condition is regarded as a variant of retinitis pigmentosa and arises due to either failure of normal development of the outer rod and cone photoreceptor segments or degeneration subsequent to initial retinal development.
- *Retinal dysplasia:* This condition can occur in isolation in an otherwise normal eye or as part of a syndrome, for example, Norrie's disease, trisomy 13, incontinentia pigmenti, Peter's anomaly, cyclopia, synophthalmia, maternal D-lysergic acid diethylamide (LSD) consumption, and the HARD+/–E (hydrocephalus, agyria, retinal dysplasia +/– encephalocele) syndrome. There is abnormal organization of the retinal cell layers that is characterized by the presence of tubular retinal folds composed of rosettes formed from one or more cell layers of photoreceptors arranged around a central lumen, at the perimeter of which is a structure similar to the retinal external limiting membrane (Fig. 31.12).
- *Retinoblastoma:* Retinoblastoma is the commonest tumor of the retina. As the name implies, the histological appearance resembles that of the developing retina. About 9% of retinoblastomas are congenital. These are mostly familial cases, which comprise approximately 40% of retinoblastomas overall. Retinoblastoma is frequent in 13q deletion syndrome, whether this is complete or an interstitial deletion involving

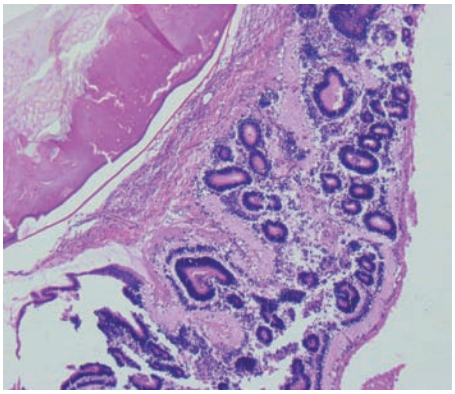

FIGURE 31.12. Retinal dysplasia. The retina is thickened and disorganized with multiple rosettes of primitive photoreceptor cells.

the 13q14 locus. Characteristic midfacial dysmorphism has been described with an interstitial deletion (Motegi et al. 1983).

- *Congenital nonattachment of the retina:* In this rare disorder there is either partial or complete failure of fusion of the inner and outer layers of the optic vesicle with the intervening void forming a congenital subretinal space.
- *Congenital falciform retinal fold:* This occurs as both a sporadic and an autosomal recessive disorder in which a fold of retina extends from the optic nerve head anteriorly toward the ciliary body. The fold most commonly occurs in the inferior temporal quadrant and may be associated with persistence of remnants of the hyaloid vessels. Histological sections through the fold show the presence of dysplastic retinal rosettes.
- *Macular coloboma:* These are usually sporadic, although an autosomal dominant variant is recognized that shows bilateral exaggerated depression of the macula. There is severe visual impairment.
- *Lange's retinal fold:* This artifact of histological preparation seen in the eyes of fetuses and infants is due to detachment of the peripheral retina, close to the ora serrata, between the outer photoreceptor layer and the pigment epithelium.

Vitreoretinal Disorders

X-linked retinoschisis (congenital vascular veils in the vitreous) is a disorder resulting in schisis of the retina between the ganglion and nerve fiber layers, resulting in vitreous veils and hemorrhages. It has a worldwide prevalence of between 1 in 5000 and 1 in 25,000. There are two variants, the foveal and peripheral types. Only the peripheral variant, usually located in the inferotemporal retina, presents in the neonate. The condition is often bilateral and, in a minority of cases, there is coexistent retinal detachment. Over 100 disease-causing mutations of the responsible gene, *XLRS1*, have been described (Tantri et al. 2004).

Familial Exudative Vitreoretinopathy

This disorder is characterized by ocular abnormalities due to avascularity of the peripheral retina, resulting in retinal exudates, tractional detachment, and retinal folds and breaks. Clinically, it may be indistinguishable from retinopathy of prematurity (ROP), which probably accounts for reports of ROP in mature infants, and X-linked retinoschisis. Dominant inheritance is usual but an X-linked form is also described. Mutations in the *frizzled-4* gene, localized to 11q13–23 and encoding Wnt receptors, have been linked to autosomal dominant familial exudative vitreoretinopathy (Robitaille et al. 2002). Norrie disease gene mutations have been described in an X-linked form, indicating that they may be allelic variants (Tantri et al. 2004).

Disorders of Ocular Pigmentation

The melanin pigment-containing cells of the iris and retinal pigment epithelium may give rise to localized pigmentary conditions or form part of the spectrum of systemic disorders of pigmentation. Since the intensity of pigmentation of the iris and retina continues to deepen postnatally, only those conditions that may be clinically apparent at birth or during the neonatal period are considered here.

Congenital Ocular Melanosis

Also known as ocular melanocytosis, this neural crest disorder affects melanocytes, which may result in either focal or diffuse increased pigmentation of the sclera, conjunctiva, iris, and ciliary body. The condition may also include the skin,

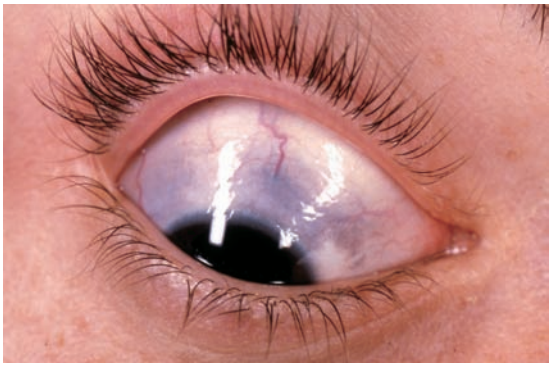

Figure 31.13. Nevus of Ota. (Courtesy of Doug Coster, Flinders Medical Centre, Bedford Park, Australia.)

when it is known as oculodermal melanosis or nevus of Ota (Fig. 31.13).

Reduced Ocular Pigmentation

Reduced intensity of ocular pigmentation of the iris and retina may occur in isolation as sex-linked recessive ocular albinism or as part of the spectrum of changes seen in generalized oculocutaneous albinism (tyrosine-negative and -positive variants), which is inherited as an autosomal recessive trait (Tomita and Suzuki 2004). X-linked ocular albinism type 1 (OA1) is caused by mutations in the *OA1* gene at chromosome Xp22, which encodes for a membrane glycoprotein on melanosomes (Camand et al. 2003).

It is noteworthy that in partial albinism (autosomal dominant), there is normal development of ocular pigmentation, presumably because the disorder does not affect melanin pigment produced by cells originating from the optic vesicle, in contrast to those derived from the neural crest.

Disorders of Optic Nerve Development

Abnormal-appearing optic nerves take many different forms. Commonly recognized anomalies include optic nerve hypoplasia, optic disk coloboma, and optic nerve dysplasia (morning glory anomaly) (Oliver and Bennett 2004).

Optic Nerve Aplasia

This rare condition causes blindness because there is failure of development of the optic disk and retinal vasculature. Associated ocular anomalies, such as anophthalmia, may coexist. The first cranial nerves may also be absent (Fig. 31.14).

Optic Nerve Hypoplasia

This is the most common congenital optic nerve abnormality and results from a reduced number of ganglion cell axons. It causes a variable degree of visual acuity and field loss. There is a male preponderance, and funduscopy reveals a small optic disk surrounded by a corona of pallor, the so-called double-ring sign. Other ocular abnormalities may coexist. Microphthalmos, aniridia, and optic nerve hypoplasia are features of de Morsier's septo-optic dysplasia syndrome. Hypoplastic optic nerves can accompany a range of CNS anomalies and conditions or syndromes involving the pituitary gland including craniopharyngioma and hormone deficiencies.

Optic nerve hypoplasia has been observed in infants of alcoholic women (Pinazo-Duran et al. 1997). It also occurs following congenital infection and maternal diabetes. Trisomy 18 and cri du chat syndrome (5p deletion) result in optic nerve hypoplasia.

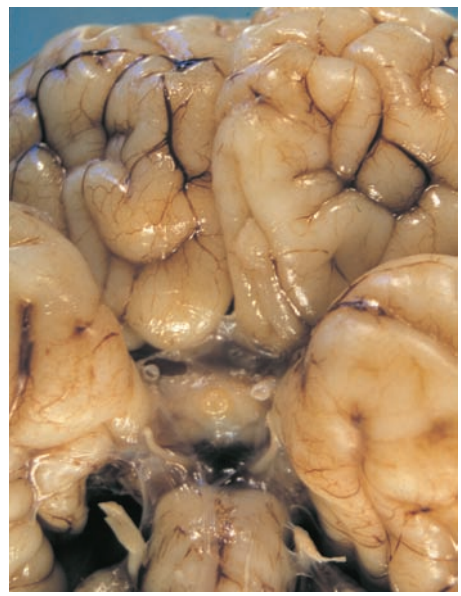

Figure 31.14. Optic nerve aplasia. Base of brain with absence of optic nerves and chiasm (same case as in Figure 31.2). The right olfactory nerve and gyrus rectus are absent. The left olfactory bulb is posteriorly sited. The pituitary stalk is present.

Optic Nerve Coloboma

The optic nerve is a relatively common site in the eye for colobomas to occur, usually causing deep excavation of the optic nerve head (in contrast to the cupping resulting from glaucoma). Variants of "typical" optic nerve colobomas include the congenital tilted disk and morning glory syndromes. "Atypical" colobomas of the optic nerve include the optic disc pit.

Retinopathy of Prematurity (Retrolental Fibroplasia)

Retinopathy of prematurity (ROP) is a condition of preterm infants with abnormal vascular proliferation affecting the retina. The clinical and pathological features of the disorder were first described by Terry (1942), who introduced the term *retrolental fibroplasia*, a reference to the retrolental gliotic mass in end-stage disease (Fig. 31.15). Retinopathy of prematurity is classified according to the International Classification of Retinopathy of Prematurity (ICROP) scale, which includes location by zone; extent of retinal involvement by clock position; stage or severity of retinopathy at the junction of the vascularized and avascular retina; the presence of plus disease, which is diagnosed when vascular dilatation and tortuosity are seen in the posterior pole; an intermediate level of plus disease (pre-plus) between normal posterior pole vessels and frank plus disease; and recognition of the more virulent form of retinopathy observed in the tiniest babies (aggressive, posterior ROP) (International Committee for the Classification of Retinopathy of Prematurity 2005). Extensive clinical studies have ratified the theme of the classification, which is that the more posterior the disease and the greater the amount of involved retinal vascular tissue, the more serious the disease (International Committee for the Classification of Retinopathy of Prematurity 2005).

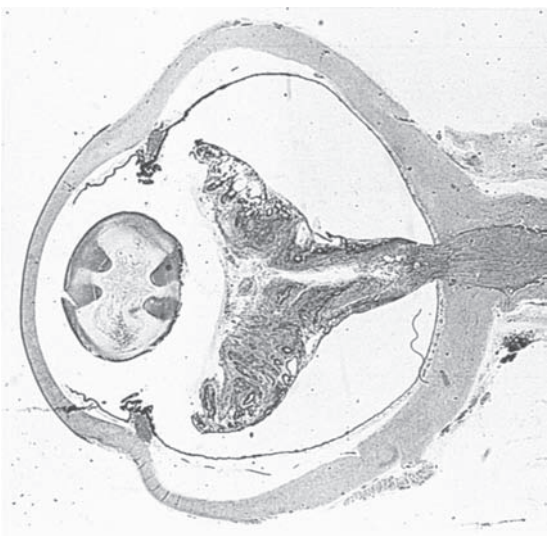

Figure 31.15. Retinopathy of prematurity. Section through eye showing retinal detachment and formation of a gliotic mass behind the lens.

The development of ROP is related to the infant's ambient oxygen concentration but other factors also contribute. Despite the use of modern monitoring techniques, it may still not be possible to eliminate ROP. Blood transfusion is implicated because the difference in oxygen desaturation between fetal and adult hemoglobin could result in delivery of extra amounts of oxygen to the ocular tissue; equally, it could be argued that infants requiring blood transfusions are generally sicker and ill infants are more likely to develop ROP. Birth weight and gestational age are risk factors.

In utero, the oxygen pressure in the fetus is considerably lower than immediately after birth. This change in oxygenation would not affect mature retinal blood vessels but may trigger vasoconstriction in the partially formed blood vessels or avascular areas of the retina in a premature infant. This would lead to peripheral retinal ischemia and set off a cascade that would cause ROP (Good and Gendron 2001). The molecular events, based largely on experimental studies, include upregulation of angiogenic factors such as vascular endothelial growth factor (VEGF), hypoxia inducible factor-α (HIF-1α), and insulin-like growth factor-1 (IGF-1), and downregulation of homeostatic proteins such as Tubedown-1 (Good and Gendron 2001). However, the predictable timing of the onset of pre-threshold ROP based on the postmenstrual age of the infant would suggest that factors that are present from the time of conception may play a significant role in ROP progression (Good and Gendron 2001; International Committee for the Classification of Retinopathy of Prematurity 2005).

A population-based study found that the severity of disease, in terms of extent, location, and plus disease, was associated with the degree of prematurity, being more severe in the most premature. Severe visual impairment affected 13% of those who were followed up at 1 year, indicating that retinopathy of prematurity remains a significant cause of severe disability among survivors of neonatal intensive care (Haines et al. 2005). Further deterioration in visual structure and function in children with severe ROP can occur in later childhood, even in children with relatively good structural findings at 10 years of age (Palmer et al. 2005). Complications of ROP include myopia, unfavorable optotype acuity, strabismus, anisometropia, amblyopia, retinal detachment, glaucoma, and cataract (Good and Gendron 2001).

Infection and the Eye

The eye is susceptible to infection by a range of microorganisms. Maternal viral infections can affect the eye both during the period of organogenesis and later in development. The eye is susceptible to maternal systemic infections such as *Toxoplasma gondii* and *Treponema pallidum* in late pregnancy, and ascending infection can also involve the eye. Ocular infection may occur during birth or in the neonatal period.

Intrauterine Infection

Antenatal infection characteristically results in a chorioretinal scar or an active chorioretinitis (Mets 2001). These are typically viral such as cytomegalovirus (CMV), herpes simplex virus (HSV), lymphocytic choriomeningitis virus, and varicella zoster infections or protozoan such as congenital toxoplasmosis. Congenital cataracts are suggestive, but less specific for congenital infection. They may be a relatively isolated finding in rubella, syphilis, varicella zoster, and Epstein-Barr virus infections. When they are present in congenital toxoplasmosis, HSV, and CMV, they are associated with extensive eye involvement (Figs. 31.16 and 31.17). These agents appear to be both a direct toxic and a teratogenic effect. Epidemiological studies suggest that some infections, such as parvovirus and influenza infection, can cause anophthalmos and microphthalmos (Busby et al. 2005).

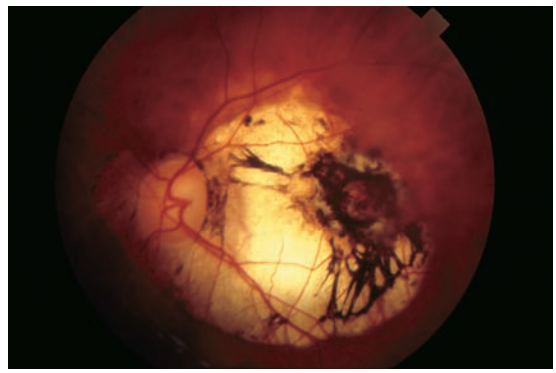

FIGURE 31.16. The fundus in quiescent congenital toxoplasmosis. (Courtesy of Doug Coster, Flinders Medical Centre, Bedford Park, Australia.)

Neonatal Infection

Infection manifest during the neonatal period may have its genesis during the delivery by infection following membrane rupture or during birth. Prolonged membrane rupture (Iroha et al. 1998) and maternal vaginitis (Isenberg et al. 1996) increase the risk of ophthalmia neonatorum. This is a purulent conjunctivitis occurring during the first 10 days of life. *Chlamydia trachomatis* is numerically the most important organism. Other organisms are *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Candida albicans* (Di Bartolomeo et al. 2001). Fecal contaminants of the maternal genital tract,

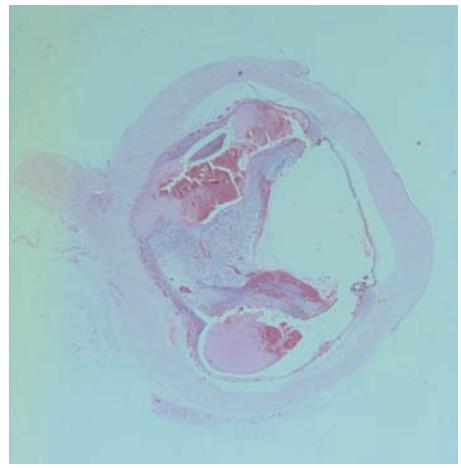

FIGURE 31.17. Congenital toxoplasmosis. There is an extensive organizing retinal exudate and retinal detachment.

streptococci, and pneumococci, have also been associated with ophthalmia neonatorum. Herpes simplex virus type 2, usually acquired during delivery but also in late pregnancy, can result in keratoconjunctivitis and endophthalmitis with retinal necrosis. Intranuclear inclusions are seen on light microscopy. Neonatal herpes infection is frequently fatal, but survivors may develop cataract and retinal scarring.

Risk factors for hospital-acquired neonatal conjunctivitis are low birth weight, ventilator or nasal cannula, continuous positive airway pressure, and ophthalmological examination (Haas et al. 2005). Clinical conjunctivitis, defined as disease necessitating treatment with or without culture, is more prevalent than culture-proven conjunctivitis. Among neonatal intensive care units in a developed country, coagulase-negative staphylococci, *S. aureus*, and *Klebsiella* spp. were the most frequent nosocomial infections.

Trauma

Periorbital and ocular trauma has been described following amniocentesis, episiotomy, electronic fetal monitoring, and vaginal delivery, especially instrumental delivery (Fig. 31.18). An incidence of ocular injuries of 12% of 2000 consecutive births has been reported (Jain et al. 1980). Retinal and vitreous hemorrhages are reported most often, but more serious injuries including hyphema, Purtscher's retinopathy, and optic nerve injury have also been reported (Khalil et al. 2003).

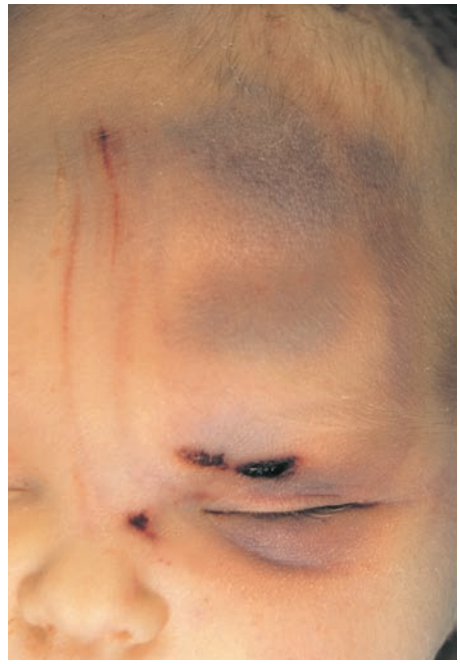

Figure 31.18. Birth injury. There is extensive orbital and periorbital bruising and excoriation. The imprint of the forceps blade is clearly seen.

The Ear

The structures forming the ear act as specially adapted mechanoreceptors that are sensitive to the stimulus of sound waves (hearing) and gravitational force (balance). The organs responsible are the organ of Corti in the cochlea and the vestibular apparatus, which are both situated in the inner ear. Functionally, the ear is composed of three compartments: (1) the external ear, formed from the sound-collecting pinna and the auditory canal; (2) the middle ear, which converts sound waves into mechanical energy; and (3) the inner ear containing the vestibulocochlear apparatus.

Embryology

The development of the ear incorporates contributions from surface ectoderm, endoderm, and the first two branchial arches, and is most easily understood by considering the development of each compartment separately (see Fig. 31.1). Understanding the regulatory mechanisms in the development of the ear has relied mainly on studies in other taxa such as birds or on studies of genetically manipulated mammals.

The External Ear

The auricle develops during the 5th week gestational age from fusion of six auricular hillocks, which arise at the margin of the first branchial groove on the surface of the second and third branchial arches and is fully formed by the fourth month. The auricle develops around the external

meatus, which begins to canalize, the first branchial cleft at week 30.

The Inner Ear

The sensory apparatus of the inner ear develops from paired otocysts around day 25 following invagination of a thickened area of surface ectoderm, the otic placode, that develops during day 22 at the level of the hindbrain. The majority of the otocyst forms the utriculosaccular chamber from which, by the 6th week, the dorsal portion starts to form the semicircular canals of the vestibular apparatus and the ventral portion, the cochlear duct, within which develops the auditory organ of Corti, during week 11.

The vestibulocochlear ganglion, the progenitor of the eighth cranial nerve develops during the 4th week, between the otocyst and the developing hindbrain. This provides innervation for the sensory mechanoreceptors of the inner ear.

The Middle Ear

The middle ear structures develop during the 4th to 6th weeks in the first pharyngeal pouch. The ossicles are derived from cartilage of the first two branchial arches and begin to ossify at 4 months. The tympanic membrane develops from the first pharyngeal membrane.

Developmental Abnormalities of the Ear

The External Ear

Minor degrees of abnormality in the shape of the auricle and the presence of accessory auricular tags due to incomplete fusion of the auricular hillocks are quite common. They are of cosmetic rather than of functional significance. More severe anomalies are seen in 1 in 12,500 birth (Karmody and Annino 1995). Malformed, low-set ears are often seen in chromosomal abnormalities, for example, trisomy 13, trisomy 18, triploidy and some autosomal deletion syndromes. Malformed auricles are recognized as part of many malformation syndromes, and deformity is severe in mandibulofacial dysostosis (Treacher Collins syndrome), Goldenhar syndrome (Fig. 31.19), and cerebrocostomandibular syndrome (Fig. 31.20); however, descriptions of the external ear are often lacking in sufficient detail to aid in syndrome diagnosis (Hunter and Yotsuyanagi 2005). Many types of auricular deformity are illustrated in *Smith's Recognizable Patterns of Human Malformation* (Jones 1997) and by Hunter and Yotsuyanagi (2005).

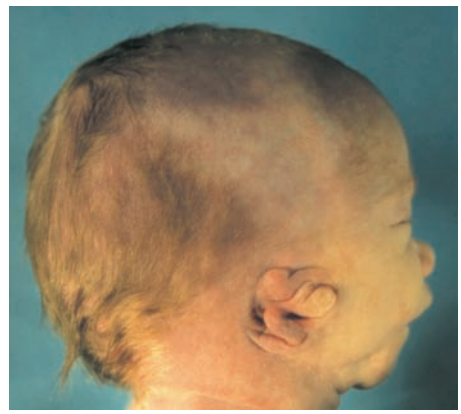

Figure 31.19. Goldenhar's syndrome. The external ear is small, low set and abnormally configured.

Microtia and *anotia* are uncommon anomalies. They are defined as malformation or hypoplasia of the auricle, ranging from a small external ear with minimal structural abnormality to total absence of the ear. Microtia is about five times more common than anotia (Mastroiacovo et al. 1995). When babies with chromosomal abnormalities are excluded, the incidence, based on

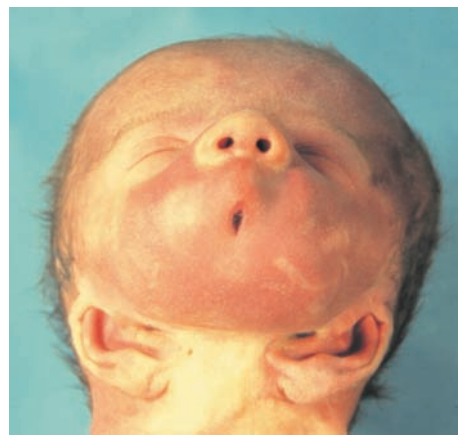

Figure 31.20. Cerebrocostomandibular syndrome. There is microstomia, hypognathia, and the ears remain below the mandible, close to the midline.

information from different malformation registers, is between 0.76 and 2.35 per 10,000 births (Harris et al. 1996). Thirty-nine percent had other malformations. There is a higher prevalence in Asians and Hispanics than in whites and blacks (Shaw et al. 2004). The commonest associated defects observed were cardiovascular anomalies, facial clefts, and anophthalmia/microphthalmia. There is a male excess in anotia/microtia (Forrester and Merz 2005), and bilateral defects are more likely in syndromic cases. Anotia/microtia is one of the commonest anomalies resulting from maternal retinoid ingestion (Lammer et al. 1985).

Congenital atresia of the external auditory canal in the absence of microtia is rare, affecting 1 in 10000 infants. The atresia may be associated with the first arch syndrome. Partial duplication of the external auditory canal may predispose to later development of branchial cleft cysts and fistulae. Defective development of the first pharyngeal pouch predisposes to atresia of the external auditory meatus and canal or malformations of the tympanic cavity and middle ear (Lambert and Dodson 1996).

Accessory tragus is a fairly common congenital malformation of the external ear. In the vast majority of cases it is an isolated developmental defect not associated with other abnormalities. However, the remote possibility exists that it could be associated with other abnormalities of the first and second branchial arch. Accessory tragus is a consistent feature of the oculoauriculovertebral syndrome (Goldenhar syndrome) (Jansen et al. 2000).

The Middle and Inner Ear

Conductive and sensorineural deafness result from abnormal development of the ossicle or organ of Corti or its neural innervation. Isolated congenital deafness is most commonly due to an autosomal-recessive inheritance. Other causes include intrauterine rubella infections before the 19th week, congenital CMV infection, maternal ingestion of streptomycin and thalidomide, severe intrapartum birth asphyxia, and postpartum hyperbilirubinemia. Very low birth weight infants are at increased risk of deafness due to hemorrhage around the eight cranial nerve. More detailed classifications of inner ear malformation (Ormerod 1960; Suehiro and Sando 1979) and teratogens affecting hearing are provided elsewhere (Dyer et al. 1998).

Michaels and Hellquist (2001) provide a useful alphabetical index of inner ear malformations and other associated conditions.

Examination of the Middle and Inner Ear

The examination of the middle and inner ear structures in detail is beyond the scope of the present chapter; the reader is referred to Michaels et al. (1985).

Syndromes Affecting Oculogenesis and Otogenesis

Oculogenesis and otogenesis occur during the critical period of organogenesis when teratogenic effects of both environmental and genetic disorders may cause severe derangement of both eye and ear development. Conditions in which congenital abnormalities of these special senses coexist include Alport syndrome, Alstrom syndrome, Crouzon (craniofacial dysostosis) syndrome, Goldenhar syndrome, Hallgre syndrome, Leber's congenital amaurosis, Norrie disease, Usher syndrome, Wallenburg syndrome, and fetal rubella syndrome and cyclophosphamide.

Olfaction

The special sense of olfaction is a phylogenetically ancient chemoreceptor-mediated function of specially adapted bipolar cells situated in the nasal mucosa.

Embryology

The olfactory bipolar chemoreceptors develop from surface ectoderm as areas of thickening referred to as the nasal placodes, which migrate toward the superior region of the developing nasal cavities where they form the olfactory pits. Further differentiation of the ectoderm occurs from these pits, resulting in the formation of the pseudostratified columnar ciliated olfactory epithelium. The olfactory bipolar cells develop synapses, via their axons, with the olfactory bulbs. At birth, the olfactory neuroepithelium is present between the

upper half of the nasal septum, the superior turbinate, and the roof of both nasal cavities. Between infancy and adulthood the extent of distribution of the neuroepithelium becomes more restricted (Nakashima et al. 1984).

Congenital Disorders of Olfaction

Magnetic resonance evaluation of patients with anosmia/hyposmia demonstrated absence or hypoplasia of olfactory bulbs and tracts in all those examined (Yousem et al. 1996).

Histopathological studies on individual olfactory deficits are rare. However, Douek et al. (1975) described histological changes in the olfactory mucosa in a variety of conditions causing anosmia, including congenital anosmia. In this condition the authors described incomplete or incorrect synapse formation between the olfactory bipolar neurons and the olfactory bulb. The olfactory epithelium is either absent or greatly reduced in congenital anosmics compared to patients who lose their sense of smell following trauma or a viral infection. In Kallmann syndrome, consisting of idiopathic hypogonadotropic hypogonadism and anosmia, mutations in the X-chromosomal KAL1 (Xp25) or in the fibroblastic growth receptor 1/KAL2 (8p12) genes lead to agenesis of the olfactory neurons (Karges and de Roux 2005). The former, X-linked, is associated with unilateral renal agenesis while the latter, an autosomal dominant form of the disease, is associated with cleft palate and dental agenesis (Albuisson et al. 2005).

Patients with Bardet-Biedl syndrome (BBS), which encompasses retinal degeneration, truncal obesity, renal and limb malformations, and developmental delay, have partial or complete anosmia. This may be the result of dysfunction of basal bodies and ciliary defects (Kulaga et al. 2004).

Congenital syphilis may cause extensive disruption of nasal development, including the olfactory mucosa.

Examination of the Nose

A description of examination of the nose and paranasal sinuses at necropsy is given by Michaels and Hellquist (2001). Formation of the nose is dependent on development of the forebrain. External examination of the nose may reveal major anomalies. In some syndromes, the nose may have distinctive features (Table 31.5) (Cohen 1993).

Table 31.5. Nasal anomalies

Nasal anomaly	Features and related anomalies
Arhinia	Absence of nose; blind dimple may be present; sporadic; extremely rare
Unilateral arhinia	One nostril absent or associated with blind dimple, skin tag or proboscis; sporadic; extremely rare
Choanal atresia	Lack of posterior opening between nasal cavity and nasopharynx—probe cannot be passed beyond 32 mm into nostril; may be associated with syndromes, e.g., CHARGE, Treacher Collins, Crouzon, Pfeiffer
Small nose	The nose is rarely measured; small nose with anteverted nares seen in de Lange, Robinow, Williams, Pfeiffer syndromes; small nose with accentuated junction between alae nasi and tip of nose seen in warfarin fetopathy; underdevelopment of alae nasi seen in Apert syndrome
Proboscis	Blind-ended, tube-like structure; multiple causes and pathogenesis; may be associated with holoprosencephaly, e.g., Trisomy 13, 18, Meckel

Nasal Tumors

Nasal Glioma

Nasal glioma, or nasal glial heterotopia, is a rare developmental abnormality that usually presents in the perinatal period as a midline mass in the nasal cavity. There may be associated hypertelorism. Histologically, the masses are composed of astrocytes (including gemistocytic type) and neuroglial fibers intermixed with a fibrovascular connective tissue stroma (Penner and Thompson 2003). Some intranasal gliomas are connected to the dura through the cribriform plate of the ethmoid bone by a fibrous stalk.

Other neurogenic developmental abnormalities are encephaloceles and meningoceles. Both are continuous with the cerebrospinal fluid (CSF) space, but, in addition, the encephalocele contains brain tissue (Hoving 2000). They can alter in size and appearance depending on the extent of CSF connection, and may be pulsatile and expand with raising of the intracranial pressure, such as during crying (Lee and Koltai 2003).

Nasal Dermoid

Nasal dermoids are benign tumors derived from ectodermal and mesodermal elements. They are midline and may present as an external nasal mass, intranasal mass, dermoid sinus without a cyst, a dermoid cyst without a fistula, or an extracranial/intracranial mass.

Taste

At birth the taste buds are present as specialized chemoreceptors formed by modification of the oral cavity surface epithelium, with the highest density of taste receptors present on the anterior two thirds of the tongue. Lower densities are also distributed over the posterior third of the tongue, the inferior surface of the soft palate, the epiglottis, and the oropharynx. The epithelium is mainly derived from the endoderm covering the medial aspect of the four pharyngeal arches. The developing taste receptors establish synaptic connections with the following nerves depending on their location and the pharyngeal arch the endoderm originated from: the lingual branch of the trigeminal nerve (anterior two thirds of the tongue, first arch), the inferior ganglion of the glossopharyngeal nerve (third arch–derived endoderm), and the inferior ganglion of the vagus (fourth arch derivatives).

Unlike the olfactory chemoreceptors, the specialized taste receptor cells are continually being desquamated and replaced. The density of taste-sensing cells is greatest in infancy and diminishes with advancing age.

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