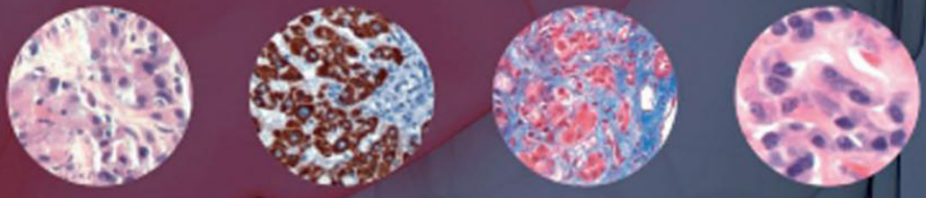
JAY H. LEFKOWITCH

SCHEUER'S Liver Biopsy Interpretation



TENTH EDITION

ELSEVIER

This 10th Edition is dedicated to the late Donald West King, M.D.
Teacher, Mentor, Student of science and the history of medicine

SCHEUER'S

Liver Biopsy Interpretation

TENTH EDITION

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New York

ELSEVIER

First edition 1968
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Preface

In the seemingly few short years since the previous edition of this book, there have been dramatic changes in hepatology and in the practice of liver pathology. Probably the most striking of these changes is the disappearing culture of the liver biopsy for chronic hepatitis C. Formerly a staple of daily biopsy reporting sessions, biopsies from patients with chronic hepatitis C for grading and staging have all but disappeared with the advent of direct-acting antiviral agents and the potential for sustained viral response and cure. With a global prevalence of some 71 million HCV-viraemic individuals, the World Health Organization initiative of 90% reduction of new or current HCV infections by 2030 is possibly reachable, but the reality is that global diagnosis of hepatitis C virus infection and access to antiviral drugs are not universal and highly dependent on variations in economies and in public health policies throughout the world. Another change since the ninth edition of *Scheuer's Liver Biopsy Interpretation* is the changed nomenclature of the disorder PBC: formerly primary biliary cirrhosis, PBC is now 'primary biliary cholangitis'. The original name 'primary biliary cirrhosis' hailed from 1950, and as morphologists, liver pathologists in particular know that the early and progressive stages of this disease have little to do with cirrhosis, and that it may take decades before cirrhosis actually develops. And, as the late Professor Sheila Sherlock advocated, relieving PBC patients of the burden and stigma of having a condition with the term 'cirrhosis' embedded in it is a good thing.

Genomic medicine looms large in pathologists' practices these days, and in liver pathology this constitutes the third of the dramatic changes in our practices since the ninth edition of this book. We are now all too frequently asked to pursue genomic evaluations of liver specimens in order to identify mutations with new or known treatment options. Consequently, those who examine liver biopsies should have a fundamental knowledge of known genomic changes among common liver tumours. To this end, there is now augmented coverage of genomic correlates throughout this 10th edition and specifically in Chapter 11 (Neoplasms and Nodules).

Despite the new or refocused directives cited above, the goal of the 10th edition is, as before, to be a practical and concise 'bench book' for use at the microscope. As Professor Scheuer wrote in the Preface to the 3rd Edition, 'the main purpose of this book, to help those who need to interpret liver biopsies', remains unaltered. By introducing new text coverage and new photomicrographs I hope this edition will provide the proper foundation by which to address the specific diagnostic questions we face in liver pathology today.

Jay H. Lefkowitch

Peter J Scheuer, MD, 1928–2006.
(Photograph by Charles Manley, Columbia University.)

In memory of Peter J. Scheuer, M.D.

'a man of an angel's wit and singular learning. I know not his fellow. For where is the man of that gentleness, lowliness and affability? And, as time requireth, a man of marvellous mirth and pastimes, and sometime of as sad gravity. A man for all seasons'.

Robert Whittington (1520)

Peter J Scheuer attended the Royal Free Hospital School of Medicine in London, UK, where he later became Professor of Pathology and Chairman of the Department of Histopathology. The first edition of Professor Scheuer's *Liver Biopsy Interpretation* was published in 1968, only a decade after Menghini first introduced the technique of needle liver biopsy. Professor Scheuer's many publications on hepatobiliary disease included seminal papers on primary biliary cirrhosis, histological grading of hepatic iron and the classification of chronic hepatitis. He collaborated extensively with his esteemed colleague Professor Dame Sheila Sherlock and the clinical Liver Unit, further establishing the Royal Free Hospital as a major international destination for patients with liver disease and for trainees in clinical hepatology and liver pathology.

N.B. For a brief history of Hepatology at The Royal Free Hospital, London, see *Campollo O. 50 years of Hepatology: The Royal Free Hospital School of Hepatology. Ann Hepatology 2020; 19: 113–116.*

Acknowledgements

Over 50 years ago, Peter Scheuer wrote the first book on liver biopsy interpretation based upon what was then a decade of microscopic experience with needle liver biopsies. This 10th edition notably contains many blocks of the original text as he wrote it back in 1968, still as accurate and concise as if newly minted. My first acknowledgement, therefore, must be of the many enduring wisdoms he embedded in the first edition of *Liver Biopsy Interpretation* that continue as important guideposts of today. I was most fortunate to have Peter Scheuer as my mentor during my fellowship training, and thereby also to have the unique opportunity of working in proximity to Professor Dame Shelia Sherlock, a formidable presence in clinical hepatology. Their luminous contemporary, if slightly more senior, colleague, Professor Hans Popper, completed the trinity of superb teachers upon whom I relied for my education in liver disease and to whom I am forever indebted.

Closer to home, my colleagues in the Pathology Department who are involved with the liver pathology service provide a stimulating and intellectually challenging environment in which to work, always bringing intriguing diagnostic questions to the table. Our superb histology technical staff, led by Sunilda Valladares-Silva, and our immunohistochemistry staff under Yuis Jimenez-Cortez consistently impress us with the outstanding quality of their work. Academic and diagnostic life for me at Columbia is greatly enriched by having colleagues like Drs Lorna Dove, Elizabeth Verna, Paul Gaglio and Alyson Fox, whose expertise in hepatology and devotion to their patients and to teaching are in a special class altogether. I must also thank those pathologists and hepatologists at other institutions who have requested my opinion on liver biopsies. These cases are never less than intriguing and provide a forum for continuing education for me, as well as for those who have asked for my input.

Michael Houston has been the major guiding force at Elsevier for *Scheuer's Liver Biopsy Interpretation*, and I am greatly appreciative of his enthusiasm for bringing out this 10th edition. I am grateful to once again be working with Joanne Scott, Deputy Content Development Manager, whose expertise in textbook publication and rigorous attention to detail and deadlines provide an ideal working environment. Thanks also to Beula Christopher King, our Project Manager in Chennai for the 10th edition, for her creative work.

Lastly, I must mention the death in October 2018 of Dr Donald West King, M.D., the former Chairman of the Department of Pathology at Columbia's College of Physicians and Surgeons. Dr King, who was my mentor from medical school through residency and beyond, was singlehandedly responsible for my setting out on a career as a liver pathologist and made my fellowship under Peter Scheuer in London possible. In so many ways, each new edition of *Scheuer's Liver Biopsy Interpretation* has served as a testament to the importance of understanding and elucidating the 'new' in biology: 'new' pathogenetic pathways, 'new' morphologic correlates of disease. Understanding the 'new' was the way of life for Dr King.

CHAPTER 1

General Principles of Biopsy Assessment

Introduction

Liver biopsy is one of many diagnostic tools used in the evaluation and management of patients with liver disease. It continues to play an important role because the concepts and classifications of liver disease are rooted in morphology. Moreover, looking at a liver biopsy specimen through the microscope is a very direct way of visualising the morphological changes that affect the liver in disease. The pathologist's interpretation (rather than mere enumeration) of these changes is used to answer important clinical questions such as disease causation and activity, and is important in therapeutic decision making.[1] A thorough and informed interpretation of liver biopsy findings therefore stands to have substantial impact on patient care. It bears emphasising that the evidence base[2] for much of liver biopsy interpretation rests on the large body of important observations reported in the pathology literature during the past 60 years since Menghini in 1958 first introduced the technique of percutaneous needle biopsy.[3] Questions of a more basic pathobiological nature can also be addressed by applying contemporary techniques of molecular and genomic medicine to liver biopsy material.

There are many reasons for performing liver biopsy (**Box 1.1**), as will be apparent from the contents of this book. In many instances in both adult and paediatric patients, the diagnosis is uncertain from clinical and radiological data, and liver biopsy provides the direct answer. Establishing a tissue diagnosis of neoplastic disease, evaluation of jaundice of uncertain cause and assessment of pyrexia of unknown aetiology are other important diagnostic problems. In the present era of emerging personalised and precision medicine, liver biopsy for tumour diagnosis (especially for hepatocellular carcinoma) optimises the possibility of genetic and molecular analysis for targeting therapy.[4,5] Pathologists are well familiar with the need for formal grading and staging of chronic hepatitis (covered in **Ch. 9**), although the availability of direct-acting antiviral therapy for hepatitis C virus has greatly reduced the volume of such biopsies. The ubiquitous 'elevated liver function tests' inscribed on biopsy requisitions are now very often explained by steatosis, steatohepatitis or related conditions (**Ch. 7**) stemming from the wide prevalence of obesity, diabetes, hyperlipidaemia and metabolic

Box 1.1 Reasons for liver biopsy

Evaluation of abnormal liver function tests
Investigation of pyrexia of unknown aetiology
Diagnosis of neoplasms
Evaluation of ascites and portal hypertension
Grading and staging of chronic hepatitis
Documentation of steatosis and its possible complications
Evaluation of liver dysfunction after liver, kidney and bone marrow transplantation
Determination of stage of fibrosis/cirrhosis for candidate who may require combined organ (e.g., heart-liver) transplantation.
Investigation of jaundice of unclear aetiology
Determination of the effects of therapy

syndrome. Indeed, in evaluating abnormal liver function tests in patients with negative serological studies, liver biopsy is rarely normal.[6] Even when normal or 'near-normal', later follow-up biopsy and/or clinical data may well provide a diagnosis of autoimmune hepatitis, primary biliary cholangitis, non-alcoholic fatty liver disease or another liver disease in a significant number of these individuals.[7–9] The workup of liver dysfunction following liver, kidney or haematopoietic cell transplantation is also reliant on information from liver biopsies, which must be reported promptly and with due consideration that the pathological changes in these patients may reflect more than one aetiological factor.

In contemporary hepatology, there are various non-invasive methods for assessing hepatic fibrosis and necroinflammatory activity which potentially might obviate the need for liver biopsy, but these methods also have recognised pitfalls.[10]

Box 1.2 Liver biopsy techniques and routes

Percutaneous
Suction (e.g. Menghini, Klatskin, Jamshidi needles)
Cutting (e.g. Vim–Silverman, TruCut needles)
Spring-loaded
Transjugular
Transgastric (endoscopic ultrasound [EUS] guided)
Thin needle with ultrasound/computed tomography guidance
Laparoscopic
Operative wedge
Fine-needle aspiration

Type and adequacy of liver biopsy specimen

Several liver biopsy techniques and routes are now available for use (**Box 1.2**), each with inherent diagnostic advantages and disadvantages.[1] Liver biopsy is an invasive technique which requires a skilled operator and all possible safeguards to minimise the risk of complications. Precise guidelines vary from one centre to another.[11] Following the biopsy procedure the needle track may be plugged with gelatin sponge (**Fig. 1.1**) or other materials[12] (**Fig. 1.2**) to prevent bleeding.[13] The standard percutaneous suction needle biopsy popularised by Menghini[3] continues to be in active use, while biopsy samples obtained with thin needles under computed tomography guidance and by the transjugular route[14] are now seen more often. Transjugular needle core biopsies occasionally are suboptimal because the specimen contains more vein wall than liver parenchyma (**Fig. 1.3**). Transgastric liver biopsy obtained during endoscopic ultrasonography is an alternative method (**Fig. 1.4**) that is useful in the diagnosis of hepatic masses and for certain lesions that are inaccessible by the percutaneous route.[15] Whatever method is chosen, the operator should carefully consider whether the specimen obtained is likely to be adequate for the intended purpose. For example, a small needle specimen obtained with a small-bore needle guided by ultrasound imaging may be adequate for the diagnosis of hepatocellular carcinoma, but not necessarily suitable for the diagnosis and histological evaluation of chronic hepatitis.[16] With needles of the Menghini type the biopsy core is aspirated and may fragment if the liver is cirrhotic. This is discussed further in **Chapter 10**. Cutting needles have been reported to produce better specimens,[17] but, in patients with focal lesions, aspiration needles often sample both the lesion itself and the adjacent liver; this is helpful in planning treatment.

Biopsy pathology differs from autopsy pathology in that there are pitfalls peculiar to small samples. A needle biopsy specimen of liver represents perhaps one fifty-thousandth of the whole organ and there is thus an obvious possibility of sampling error. Some diseases of the liver are diffuse and involve every acinus, so that sampling error is unlikely; these can be diagnosed with confidence even in small specimens. A diagnosis of acute viral hepatitis can be established in a needle specimen only a few millimetres long, whereas a specimen of similar size may not be adequate for the accurate diagnosis and evaluation of chronic liver disease, for assessment of bile duct numbers, for assessing the full extent of steatosis[18] or for the detection of focal lesions such as tumour deposits or granulomas. Focal or unevenly distributed lesions cannot be entirely excluded on the basis of their

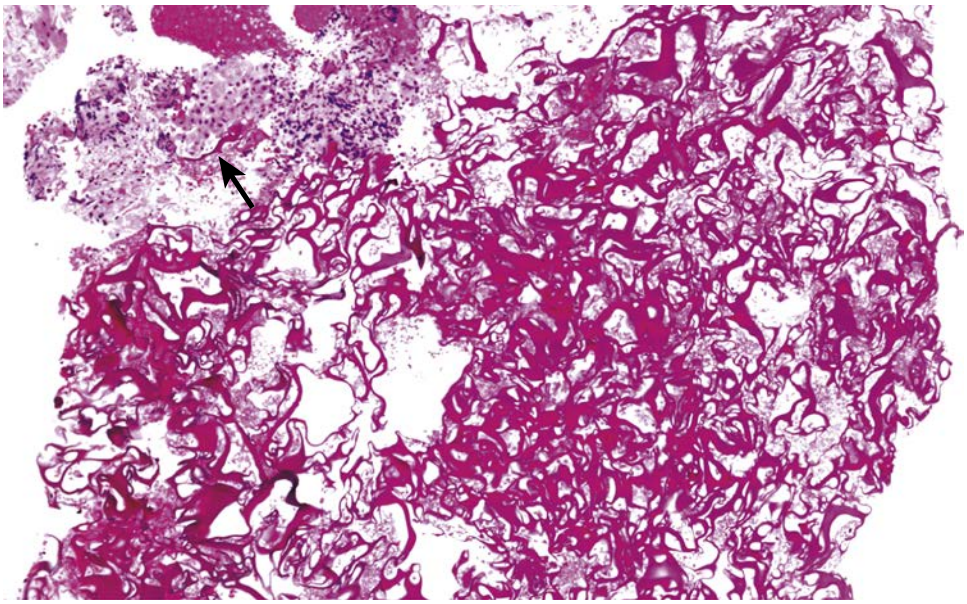

Fig. 1.1 Foreign material. This is absorbable gelatin which was used to plug a needle track. A small amount of liver tissue is seen at the point of the arrow. (Needle biopsy, H&E.)

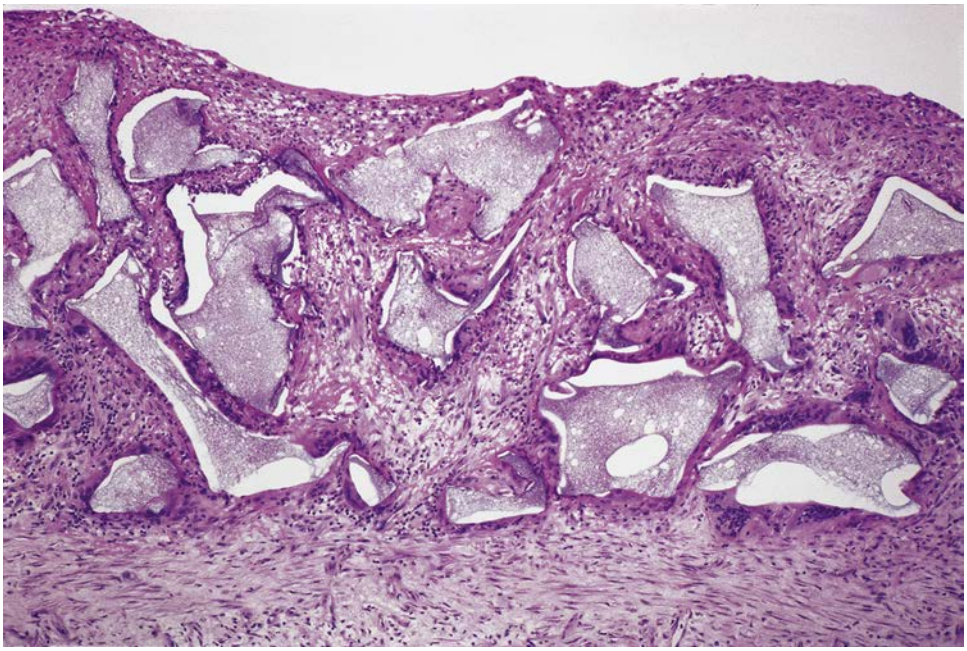

Fig. 1.2 Foreign material. Material used to plug a needle track has here escaped and produced a peritoneal foreign-body giant-cell reaction.[12] (H&E.)

absence from an unguided needle biopsy specimen. When focal lesions are suspected, multiple biopsies may help to reduce sampling error.

Chronic hepatitis and cirrhosis present particular sampling problems. In some patients with hepatitis there is a zone of extensive necrosis immediately adjacent to the capsule, whereas the deeper parenchyma is less severely affected. A small specimen consisting of tissue from the subcapsular zone of the liver would then give a misleadingly pessimistic

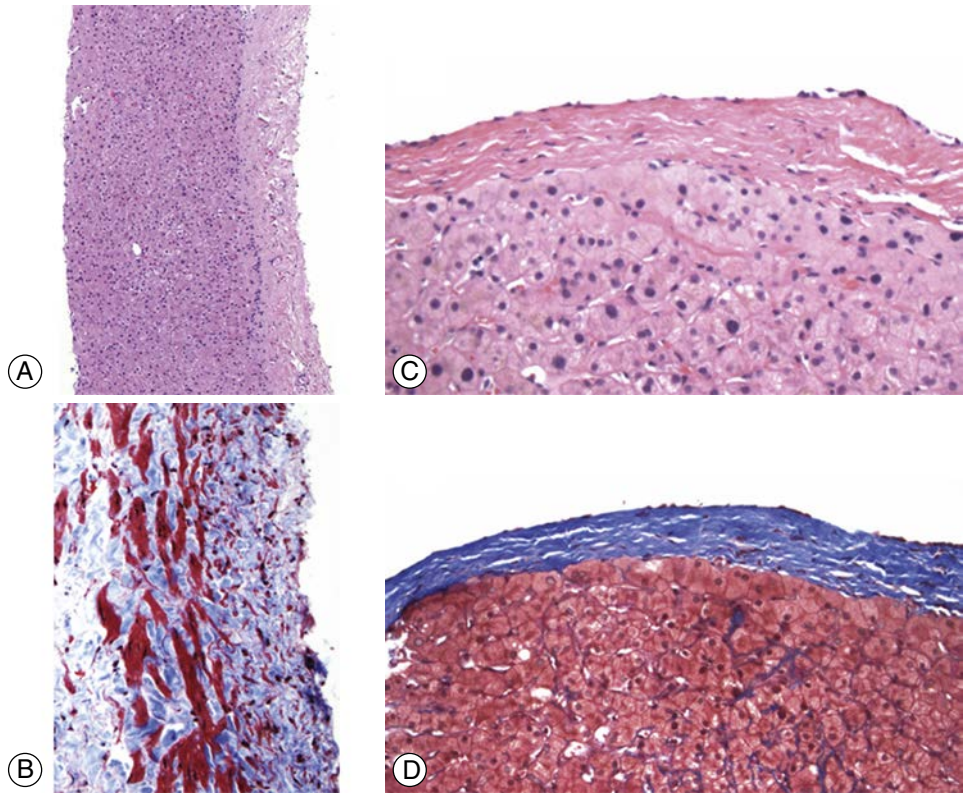

Fig. 1.3 Transjugular vs. percutaneous needle biopsy. A and B: Transjugular biopsy. **A:** This needle core was obtained by the transjugular route and only half the width of the core is liver tissue, with the remainder along the right side occupied by the wall of a large vein. **B:** The stroma of the vein wall contains muscle, in contrast to the non-muscular liver capsule. **C and D:** Percutaneous needle biopsy. **C:** Percutaneous biopsies usually have capsule at one end of the core. **D:** The capsule is composed of collagen fibres and fibroblasts, without muscle. (**A&C**: haematoxylin and eosin stain; **B&D**: Masson trichrome stain.)

impression (Fig. 1.5). In cirrhosis the structure of a nodule is sometimes very similar to that of normal liver, so that a sample consisting almost entirely of the parenchyma from within a nodule may present serious diagnostic difficulties (Fig. 1.6). These are accentuated by the resistance of dense fibrous tissue; in a patient with cirrhosis an aspiration biopsy needle may glance off fibrous septa and selectively sample the softer nodular parenchyma. For this reason, some clinicians prefer to use cutting needles in patients with suspected cirrhosis.[19]

Abnormalities in a liver biopsy may represent changes remote from a pathological lesion rather than the lesion itself. In large bile-duct obstruction, for example, the results of the obstruction are clearly seen in the biopsy sample, whereas the cause of the obstruction is usually not visible. The biopsy may be taken from the vicinity of a focal liver lesion such as metastatic carcinoma, and present one or more of a range of pathological features, often puzzling to the interpreter (Fig. 1.7). Similarly, disease elsewhere in the body may give rise to reactive changes in the liver; biopsy appearances are not normal but at the same time do not indicate primary liver disease.

Biopsies reveal lesions or diseases rarely seen at autopsy because of their relatively benign course, such as sarcoidosis. In other conditions the evolution of a disease to an end stage means that the earlier and more characteristic pathological features are rarely seen at autopsy or even at liver transplantation. In such cases liver biopsies provide valuable insights into the pathology of the disease.

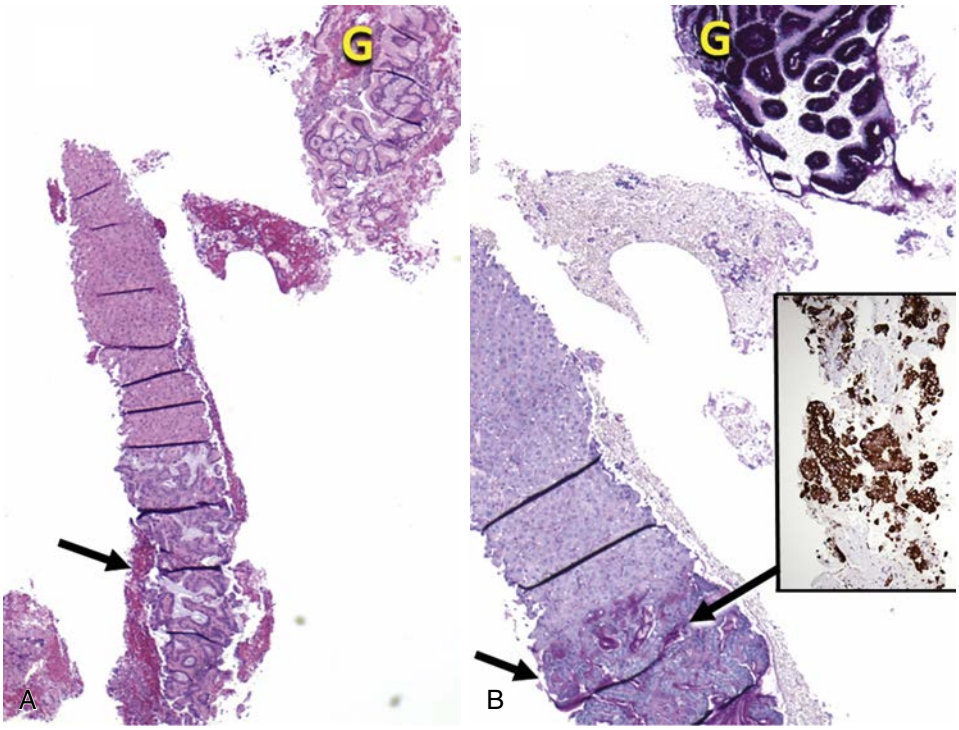

Fig. 1.4 Transgastric liver biopsy. A: The specimen includes fragments of gastric mucosa (G), blood clot and, at the bottom, a core of liver tissue infiltrated by adenocarcinoma (arrow). **B:** PAS (Periodic acid-Schiff) stain darkly stains the mucin within the gastric epithelium (G) and also within the infiltrating adenocarcinoma (arrow). Inset: The infiltrating adenocarcinoma, clinically suspicious for cholangiocarcinoma, is strongly positive on the immunostain for cytokeratin 19. (**A**: Needle biopsy, H&E; **B**: diastase-PAS; inset: specific immunoperoxidase.)

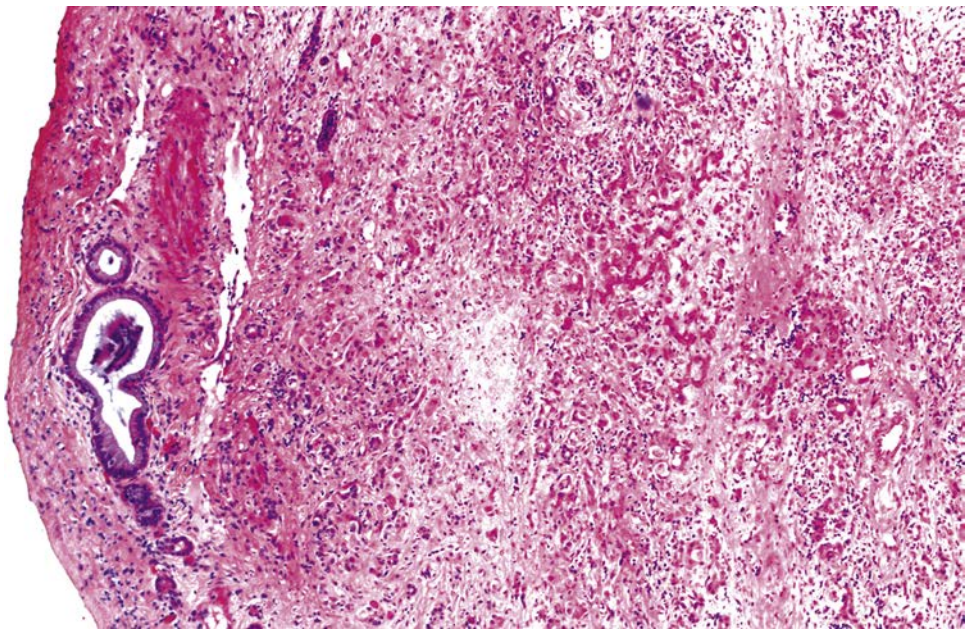

Fig. 1.5 Subcapsular necrosis. There is a zone of multiacinar necrosis immediately deep to the liver capsule (right) in this patient with chronic hepatitis. The changes are less severe in the deeper tissue to the left. A small superficial sample would have presented problems of interpretation. (Needle biopsy, H&E.)

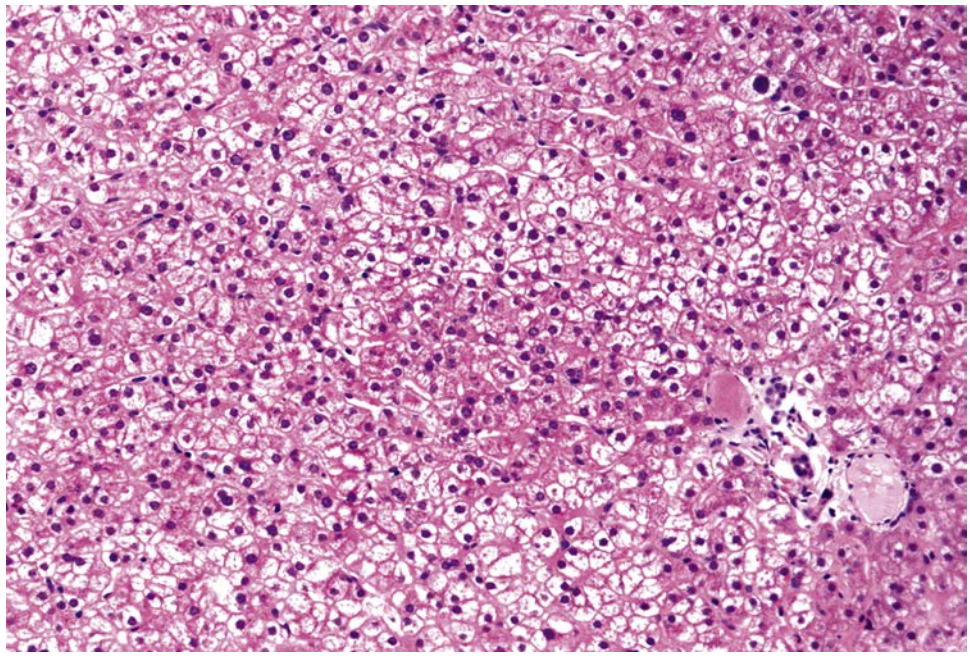

Fig. 1.6 Cirrhosis. Appearances are nearly normal because the sample is from the centre of a nodule and does not include septa. A portal tract (at right, below centre) is small and poorly formed. (Needle biopsy, H&E.)

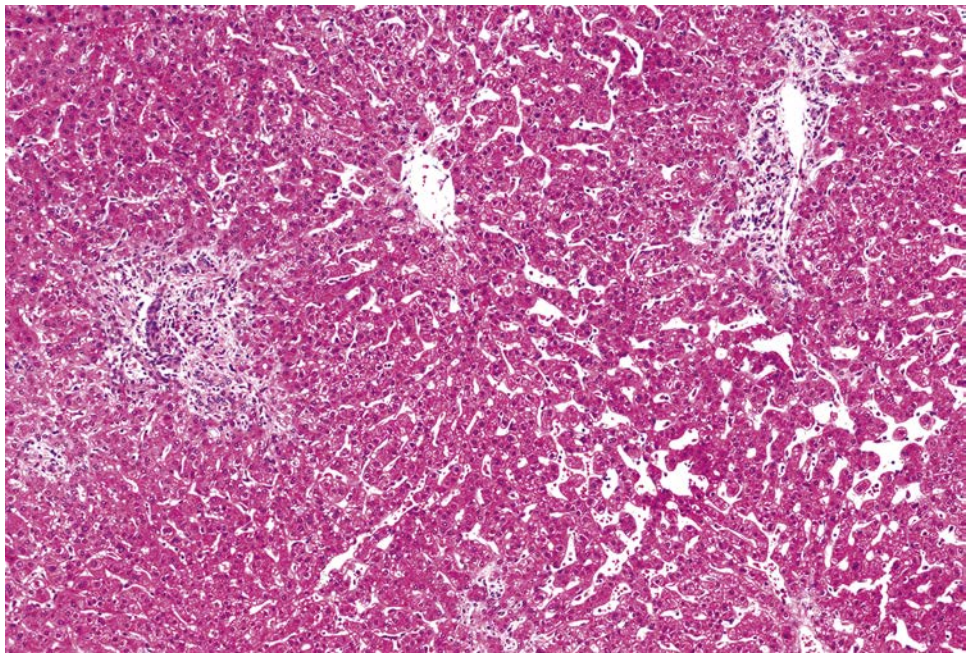

Fig. 1.7 Changes near metastatic tumour. Portal changes like those of biliary obstruction are seen (left and top right), and there is sinusoidal dilatation in the perivenular area (bottom right). (Needle biopsy, H&E.)

Liver biopsy does not always provide a final or complete diagnosis. Sometimes it even fails to give helpful information. In most cases, however, an adequate and properly processed biopsy is an important item among the diagnostic tests to which the patient is subjected. The relatively limited range of morphological reactions of the liver to injury determines a need for full clinical, biochemical, immunological and imaging data to complement the biopsy findings. Pathologists need this information in order to avoid writing clinically unhelpful, or even misleading, reports, though they may prefer to read the slides before the clinical data to avoid bias.[20] Conversely, it is important that pathologists should produce clear and full reports on the biopsy findings for their clinical colleagues. Every report should attempt to answer one or more clinical questions, whether or not these are explicitly stated on the request form. The use of a standardised checklist has been advocated as a means of ensuring that no potentially useful information is omitted.[21] However, most pathologists currently write unstructured reports. These can be supplemented by a summary giving the essential message which the pathologist wants to convey.

The specimen at the bedside and in the laboratory

Before a liver biopsy is undertaken, the clinician may wish to discuss with the pathologist the need for any special treatment of the specimen, such as freezing part of the specimen or taking tissue for electron microscopy.[20] Accurate assessment of the often subtle changes in a liver biopsy requires sections of high quality. The pathologist is usually aware of possible artefacts in liver biopsy material, as in any histological specimen. Artefacts should obviously be avoided whenever possible, and recognised as such when they do occur. A biopsy of adequate size may be made undiagnosable by rough handling (**Fig. 1.8**), poor fixation, overheating, poor microtome technique and bad staining, all of which can obscure the criteria on which histological diagnoses are based. Poor fixation coupled with prolonged saline immersion sometimes leads to potentially confusing liver-cell swelling and widespread separation of hepatocytes and distortion of the liver-cell plate structure (**Fig. 1.9**).

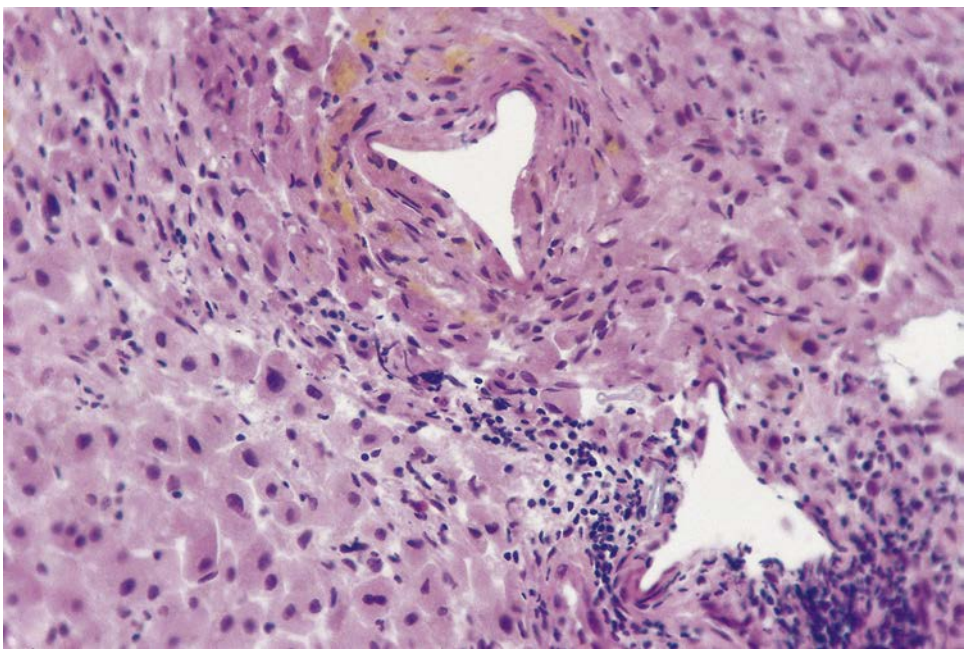

Fig. 1.8 Traumatic artefact. The triangular spaces, which slightly resemble blood vessels, are artefacts caused by rough packing of the specimen between pieces of foam sponge. (H&E.)

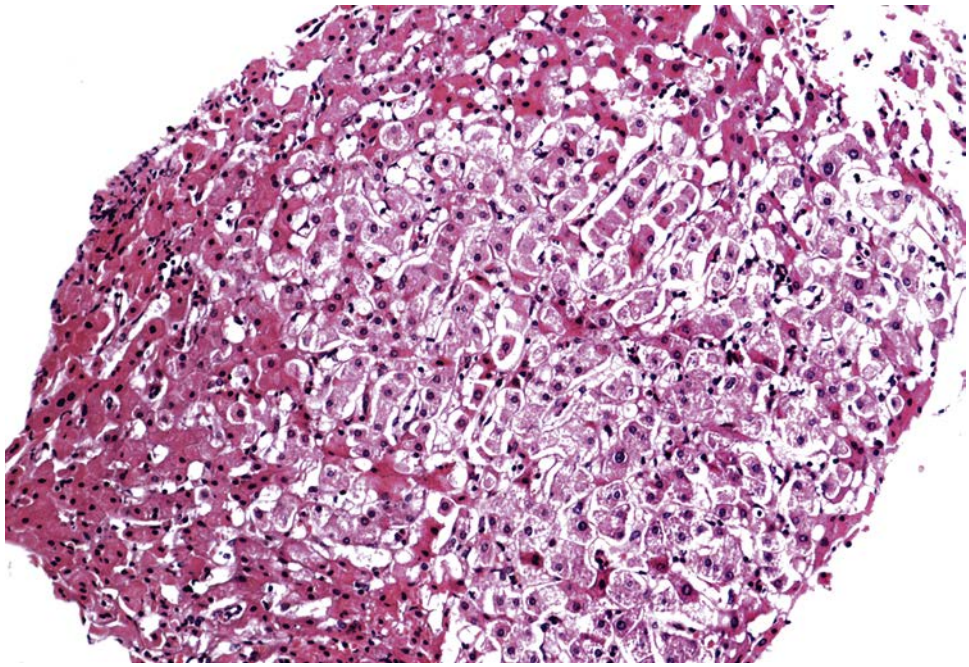

Fig. 1.9 Fixation artefact. Hepatocytes in the central part of the specimen are swollen and pale-staining because of poor fixation. Prolonged saline immersion has separated and created widened spaces between hepatocytes. (Needle biopsy, H&E.)

False-positive staining for iron is unrelated to particular cells or structures, or is in a different focal plane from the tissue. Foreign materials injected radiologically may appear puzzling to the pathologist due to unfamiliarity or because of localisation in unexpected organs which have been unintentionally embolised. Primary and metastatic tumours of the liver are often treated with drug-eluting chemoembolic gels via transarterial chemoembolisation (TACE) or by Yttrium 90 microspheres in selective internal radiation therapy (SIRT). TACE gels are large (300 μm) and typically lodge within medium-size hepatic artery branches within portal tracts,[22] while Yttrium 90 microspheres, due to their considerably smaller size (30–40 μm), may migrate from portal tract arteries to small portal microvessels, periportal inlet vessels and sinusoids[23] (**Fig. 1.10**).

This book is mainly about changes seen in conventionally stained paraffin sections and cytological preparations. There are many other ways of looking at or investigating a tissue sample, some of them helpful in routine diagnosis. Immunohistochemistry is frequently an essential aspect of liver biopsy evaluation. Its value in individual diseases is covered in the subsequent chapters. One example is use of immunostains to address the functional heterogeneity and 'zonation' of the normal liver lobule or acinus (which is based upon oxygenation[24] and Wnt/β-catenin signalling[25,26]). The liver's zonation can be demonstrated using immunohistochemical stains for enzymes localised to particular acinar zones. A striking example is glutamine synthetase, which is involved in ammonia metabolism and is only present in the several layers of hepatocytes surrounding efferent venules (**Fig. 1.11**). Demonstration of the ductular reaction using antibody to cytokeratin 7 (or 19) (**Fig. 1.12**) is important in several chronic biliary tract diseases, in fibrosing cholestatic hepatitis after liver transplantation and in the progression of fibrosis in steatohepatitis and other conditions.[27] Immunostains are useful in demonstrating viral hepatitis antigens (**Ch. 9**) and are the most accurate way of diagnosing α_1-antitrypsin deficiency morphologically. Immunohistochemistry is used extensively in the evaluation of primary and secondary tumours of the liver (**Ch. 11**). Electron microscopy has a well-defined place in liver pathology and is dealt with in the final chapter.

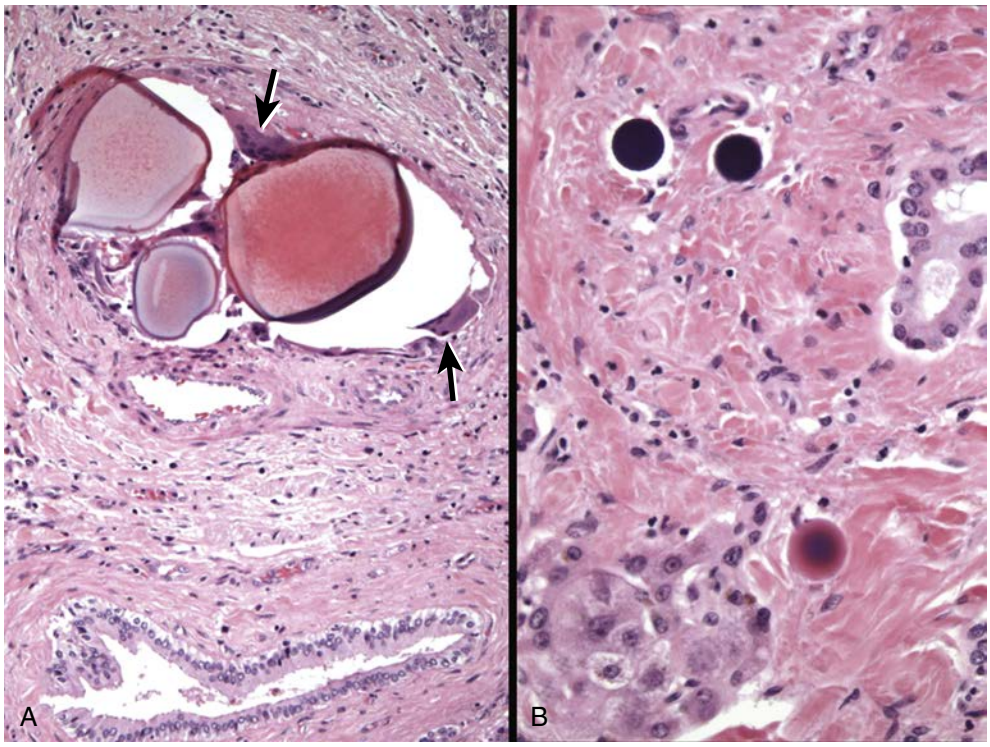

Fig. 1.10 Chemoembolic gels and Yttrium 90 microspheres. A: Chemoembolic gels are present within a medium-sized hepatic artery branch. Foreign-body giant cells have gathered around the gels (arrows). **B:** Yttrium 90 microspheres are present within microvessels in and adjacent to the portal tract. (Explant livers, H&E.)

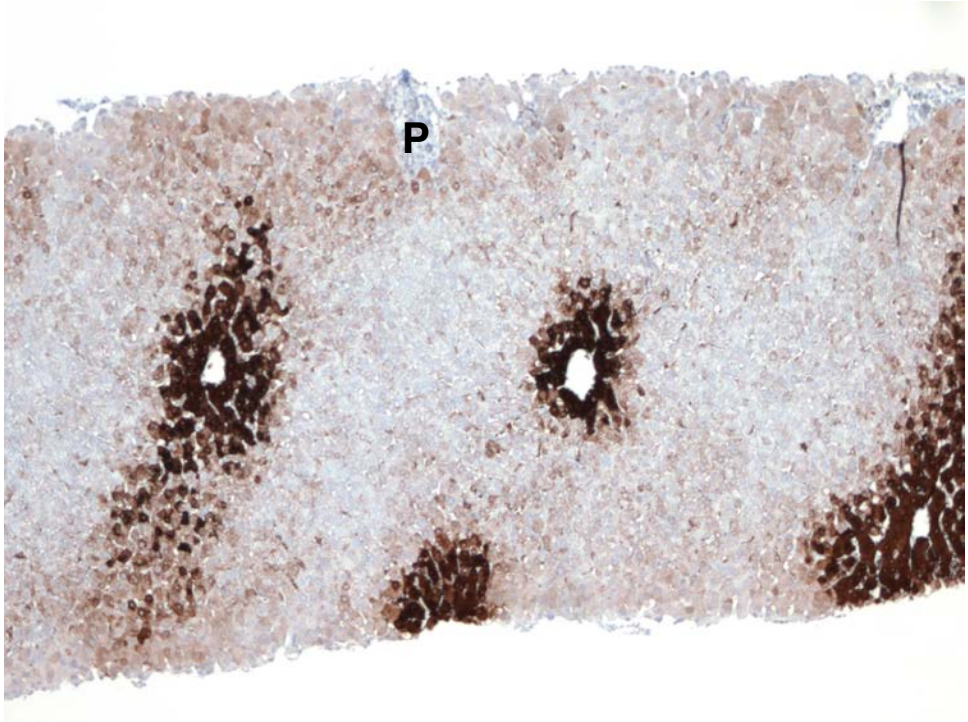

Fig. 1.11 Immunohistochemistry and the functional heterogeneity of the liver lobule. Glutamine synthetase immunostain shows positivity for this urea cycle enzyme localised to a rim of perivenular hepatocytes, while the mid-zone and periportal regions are negative. P, portal tract.

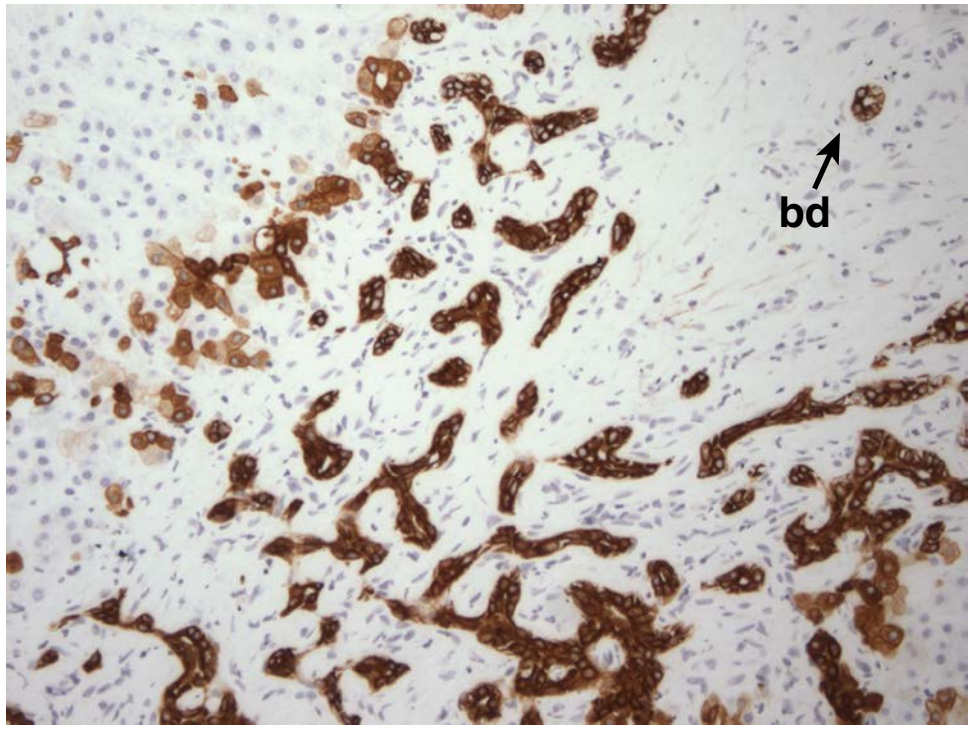

Fig. 1.12 Cytokeratin 7 immunostain in biliary tract disease. A vigorous ductular reaction has developed in this case of primary sclerosing cholangitis, as demonstrated with cytokeratin 7 immunostain. The native bile duct (bd) is also identified with this method.

In situ hybridisation has been applied to liver tissue for the identification or assessment of replication of hepatitis viruses and cytomegalovirus. The polymerase chain reaction (PCR) can be applied to liver tissue, and provides more direct evidence of virus infection in the liver than serum PCR. DNA extracted from biopsy tissue can be used in analysis of viral infections and several inherited metabolic diseases.

Part of the biopsy specimen can be analysed for copper, iron or abnormally stored substances, and enzyme activities can be assayed by micromethods. In the case of copper and iron, these measurements can, if necessary, be made after paraffin embedding, as discussed in **Chapter 14**. Elution of Sirius red from sections provides an accurate method for the measurement of tissue collagen,[28] and this stain is also used for image analysis of collagen.[29,30] *In situ* demonstration of enzymes can be achieved by immunocytochemical methods or by means of enzyme histochemistry, as has been described in the functional zonation of human liver.[31,32]

Well-established techniques of morphometry and image analysis have been applied to tissue sections to obtain data on relative volumes of tissue components in normal human liver[33,34] and in disease. Three-dimensional reconstruction using a computer has helped in the understanding of disease processes and of the relationship between anatomical structures.[35–37]

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CHAPTER 2

Laboratory Techniques

Processing of the specimen

As soon as a needle biopsy specimen is obtained from the patient, it should be expelled gently into fixative or onto a piece of glass, card or wood. Filter paper is less suitable because fibres tend to adhere to the tissue and may interfere with sectioning. The specimen must be treated with great care, and excessive manipulation should be rigorously avoided; distortion of the specimen by rough handling at this stage may seriously interfere with accurate diagnosis, because diagnosis often depends on subtle criteria. At this stage, minute pieces can be put into an appropriate fixative for electron microscopy (**Ch. 17**), preferably by an operator experienced in this technique, and samples taken for chemical analysis or freezing. Frozen sections may be needed for demonstration of lipids. If porphyria is suspected, a very small amount of the unfixed tissue can be examined under ultraviolet light or with a suitable quartz halogen source, either whole or smeared onto a glass slide.

Tissue for paraffin embedding should be transferred to a fixative as soon as possible. When transit to the laboratory is likely to involve much movement, it is helpful to fill the container to the brim with fixative. Buffered formalin and formol saline are both suitable for routine fixation, which is accomplished after 3 h at room temperature or less at higher temperatures (**Table 2.1**). Operative wedge biopsies and larger specimens need longer fixation. Fixatives other than formalin are successfully used in some centres; handbooks of laboratory techniques should be consulted for optimum times and conditions for each fixative.

Minute fragments can be hand-processed more quickly than larger pieces, and this also avoids undue shrinkage and hardening. Automated vacuum embedding allows the time of processing of needle specimens to be drastically reduced, as shown in **Table 2.1**; the ultrarapid method by which a good section can be produced in about 2 h has become important because of the need for rapid decisions on treatment in patients who have undergone liver transplantation. Frozen sections, occasionally needed for a decision at surgery, can be cut by a standard method using a cryostat. They are sometimes adequate for diagnosis of obvious lesions such as neoplasms, but are unsuitable for recognition of subtle changes, and can even be dangerously misleading.

The exact number of sections routinely cut from a block varies widely from one laboratory to another. In Scheuer's former laboratory at the Royal Free Hospital in London, 10 or more consecutive sections 3–5 μm thick are cut from each block and alternate sections used for the staining procedures outlined in the next paragraphs. The remaining sections are stored. Step sections are used when discrete lesions such as granulomas or tumour deposits are suspected or for identification of bile ducts when duct paucity is suspected. Serial or near-serial sections are helpful when utilising multiple immunohistochemical stains.

Table 2.1 Sample tissue schedules for liver biopsies.

Agent	Manual overnight automatic (vacuum)*	Routine overnight automatic	Routine automatic (vacuum)*	Ultrarapid
Buffered formalin	3 h	3 h	2 h	30 min
Formalin–ethanol–water (1:8:1)	Overnight	–	–	–
70% ethanol	–	3 h	1 h	3 min
90% ethanol	–	3 h	1 h	2 min
100% ethanol	2 × 1 h	2 × 2 h	3 × 1 h	3 × 2 min
Xylene	3 × 1 h	3 × 1 h	4 × 1 h	4 × 5 min
Wax (60°C)	2 × 1 h	2 × 1 h	3 × 1 h	3 × 5 min
Total time	24 h	18 h	14 h	1 h 16 min

*All at 50°C except for wax step.

Choice of stains

The stains routinely applied to liver biopsies vary according to local custom. The minimum advised is haematoxylin and eosin (H&E) and a reliable method for connective tissue. The author prefers a silver preparation for reticulin as the principal method for showing connective tissue, for reasons discussed below, but trichrome stains also have important applications and can reveal changes not easily seen in a reticulin, such as the pericellular fibrosis of steatohepatitis. Routine staining for iron enables the biopsy to be used to screen for iron storage disease and the periodic acid–Schiff (PAS) stain after diastase digestion (DPAS or PASD) provides a relatively crude, but practicable, screening procedure for α_1-antitrypsin deficiency as well as showing activated macrophages and bile-duct basement membranes. Stains for copper-associated protein, elastic fibres and hepatitis B surface antigen are useful (orcein and Victoria blue methods can stain all three of these) and arguably essential additions to the routine list. Rhodanine stain is an excellent method for demonstrating copper itself. Some pathologists like to see two H&E-stained sections, one from the beginning and the other from the end of a series of consecutive sections. Other methods are used as required for particular purposes. The extent to which 'special' stains form part of the routine set must be decided by each pathologist. The volume of liver biopsy specimens and the institutional resources have an impact on which set of stains is adopted.[1]

A **reticulin** preparation is important for accurate assessment of structural changes. Without it, thin layers of connective tissue and hence cirrhosis may be missed, as may foci of well-differentiated hepatocellular carcinoma in which the reticulin structure is often highly abnormal (**see Fig. 11.13**). Counterstaining is sometimes used, but is apt to distract rather than help, bearing in mind that the chief function of the reticulin preparation is to provide a sensitive low-power indicator of structural changes.

Stains for **collagen** such as chromotrope–aniline blue (CAB) are important for the detection of new collagen formation, especially in alcoholic steatohepatitis and its imitators (Ch. 7). Collagen staining is therefore advised for any biopsy showing substantial steatosis. It also helps to show blocked veins within scars; these are easily missed on H&E staining. It is therefore wise to use a trichrome stain when vascular disease is suspected.

A stain for **elastic fibres** such as the orcein stain, Victoria blue or elastic–Van Gieson is also useful to identify blocked vessels. The stains often enable the pathologist to distinguish

between recent collapse and old fibrosis, since only the latter is positive (Ch. 6). Again, this distinction may be very difficult to make on H&E and even with the help of stains for collagen and reticulin. The orcein and Victoria blue also show copper-associated protein and hepatitis B surface material.

Staining for **iron** by Perls' method or another similar technique enables not only iron but also bile, lipofuscin and other pigments to be evaluated, as discussed in Chapter 3. Counterstaining should be light to avoid obscuring small amounts of pigment.

Staining of **glycogen** by means of the PAS method or Best's carmine demonstrates the extent of any liver cell loss, and shows focal areas devoid of hepatocytes such as granulomas. **Glycoproteins** may be demonstrated by the PAS method after digestion with diastase to remove glycogen. This stain serves to accentuate hypertrophied macrophages, such as Kupffer cells filled with ceroid pigment after an acute hepatitis or episode of cholestasis. Alpha$_1$-antitrypsin bodies stain strongly, but the stain is not sufficiently sensitive to enable all examples of α_1-antitrypsin deficiency to be detected.

Staining for **copper** is mainly used in suspected Wilson's disease, although, as explained in Chapter 14, it is not always helpful and may even be negative. The rhodanine method is preferred because it is easy to distinguish the orange-red colour of copper from bile, a distinction which is occasionally difficult with rubeanic acid. In Wilson's disease, there is variable correlation between the presence of stainable copper and staining for **copper-associated protein**. In chronic cholestasis, however, the two usually correspond. Table 2.2 shows the special stains used at our institution as a regular panel for liver specimens, along with their specific utility.

Other non-immunological methods useful on occasion include the Ziehl–Neelsen stain for mycobacteria and for the ova of *Schistosoma mansoni*. Specific staining for bilirubin is rarely necessary, but conjugated bilirubin is stained a bright green colour by the Van Gieson method (**see Fig. 4.10**) and a forest green colour by Hall's stain. **Amyloid** is stained by the usual techniques.

For **immunohistochemical staining**, standard techniques are applied. Among antibodies that are helpful in everyday practice are those against components of the hepatitis B virus, the delta agent, cytomegalovirus and α_1-antitrypsin. Neoplasms of doubtful histogenesis or differentiation are investigated by appropriate panels of antibodies, as in any other organ. In hepatocellular carcinoma, bile canaliculi between tumour cells may stain with a polyclonal anti-CEA (carcinoembryonic antigen) antibody, cross-reacting with a canalicular antigen. Assessment of bile-duct loss may require staining of cytokeratins 7 and 19, characteristic of bile-duct rather than liver-cell cytoplasm and of the ductular reaction (Ch. 4). The application of immunohistochemistry and of other modern techniques is discussed in more detail in Chapter 17.

Most of the staining methods mentioned above are used routinely in many laboratories, and can be found in the books listed under 'General reading' at the end of this chapter. A selection of methods is given below (Box 2.1).

Table 2.2 Special stains in evaluating liver biopsies.

Stain	Utility in identifying specific structure(s) and/or process
Trichrome	• Fibrosis: - *Portal/periportal*: in chronic hepatitis; in chronic biliary tract disease, chronic liver allograft rejection, other conditions. - *Perivenular:* cardiac sclerosis; after central perivenulitis of allograft cellular rejection; after variant form of autoimmune hepatitis; after steatohepatitis (alcoholic or non-alcoholic type). - *Perisinusoidal:* steatohepatitis-related (alcoholic or non-alcoholic fatty liver disease) (zone 3); 'diabetic hepatosclerosis' [non-zonal]; hypervitaminosis A (diffuse); congenital syphilis (diffuse)

Table 2.2 Continued

Stain	Utility in identifying specific structure(s) and/or process
Reticulin	• Lobular architecture • Periportal regenerative hyperplasia (thickened liver-cell plates) • Nodular regenerative hyperplasia (NRH) • Reticulin collapse/condensation in acute and/or chronic hepatitis • Bridging necrosis • Lymphoid aggregates and/or follicles (twig-like in regions of lymphoid cells) • Paucireticulin pattern in hepatocellular carcinoma • Fibrosis pattern (including subtle portal/periportal fibrosis in non-cirrhotic portal hypertension, or several metabolic/storage disorders (e.g., Glycogen storage disease; Mauriac syndrome)
Iron	• Hemosiderin granules in Kupffer cells, hepatocytes, bile ducts, portal macrophages • Ferritin (bluish hue in hepatocytes) • Localize focal bile canalicular bile plugs in minimal cholestasis
PAS	• Identify glycogen in hepatocytes • Identify centrilobular hepatocyte pallor (glycogen depletion) due to hepatic hypo-perfusion or post–liver transplant 'preservation injury' (ischemia–reperfusion injury) • Helpful in identifying small and large droplet fat, due to vacuolar spaces in contrast to purple-staining hepatocyte cytoplasm • Excess hepatocellular glycogen (e.g., glycogen storage diseases—especially type 4 glycogen-storage disease; polyglucosan bodies; glycogenosis; glycogenic hepatopathy) • Lobular architecture: portal tracts and fibrous septa appear pale compared to glycogen—provides overview of architectural status
DPAS*	• Alpha$_1$-antitrypsin deficiency (periportal hepatocellular globules) • Bile duct and bile ductular basement membranes • Copper-binding protein in periportal hepatocytes • Phagocytic debris in Kupffer cells, portal macrophages, after hepatocyte necrosis, acute hepatitis and/or in portal inflammatory processes
Victoria blue	• Hepatitis B surface antigen in hepatocytes • Copper-binding protein in periportal hepatocytes • Elastic fibres in portal tracts, scars, hepatic arterioles (internal elastic membranes)
Rhodanine	• Copper in hepatocytes

*DPAS = diastase-pretreated periodic acid Schiff stain

Box 2.1 Staining methods

Silver impregnation for reticulin fibres (Gordon & Sweets)

1. Bring section to distilled water.
2. Treat with acidified potassium permanganate for 10 min; wash in distilled water.
3. Leave section in 1% oxalic acid until pale (about 1 min). Wash well in several changes of distilled water.
4. Mordant in 2.5% iron alum for 10 min. Wash in several changes of distilled water.
5. Treat with silver solution until section is transparent (about 10–15 s). Wash in several changes of distilled water.

Box 2.1 Continued

6. Reduce in 10% formalin (4% aqueous solution of formaldehyde) for 30 s. Wash in tap water followed by distilled water.
7. Tone if desired in 0.2% gold chloride for 1 min. Rinse in distilled water.
8. Fix in 2.5% sodium thiosulphate for 5 min. Wash several times in tap water.
9. Transfer section to ethanol, clear and mount.

Reticulin appears black. The colour of the collagen varies according to whether step 7 is used; in untoned preparations it is yellow-brown.

Silver solution

To 5 ml of 10% aqueous silver nitrate, add strong ammonia (specific gravity 0.88) drop by drop until the precipitate which forms is just dissolved. Add 5 ml of 3% sodium hydroxide. Add strong ammonia drop by drop until the resulting precipitate dissolves. The solution does not clear completely. Make up to 50 ml with distilled water. Scrupulously clean glassware should be used throughout.

Acidified potassium permanganate

To 95 ml of 0.5% potassium permanganate, add 5 ml of 3% sulphuric acid.

Chromotrope–aniline blue (CAB) method for collagen and Mallory bodies

(As used at Mount Sinai Hospital, New York; modified from Roque[2] and Churg & Prado[3])

1. Bring section to water.
2. Stain nuclei by the celestine blue–Lillie Mayer sequence or other method. Rinse in distilled water.
3. Immerse in 1% phosphomolybdic acid for 1–3 min. Rinse well in distilled water.
4. Stain with CAB solution for 8 min. Rinse well in distilled water. Blot.
5. Dehydrate quickly, clear and mount.

Collagen is stained blue. Mallory bodies stain blue or sometimes red. Giant mitochondria stain red.

CAB solution

Aniline blue (1.5 g) is dissolved in 2.5 ml HCl and 200 ml distilled water with gentle heat; 6 g chromotrope 2R is added. The pH should be 1.0.

Orcein stain for copper-associated protein, elastic fibres and hepatitis B surface material[4]

1. Bring section to water.
2. Treat with acidified potassium permanganate for 15 min.
3. Rinse in water and decolorise in 2% oxalic acid.
4. Rinse in distilled water, then wash in tap water for 3 min.
5. Stain in commercial orcein solution for 30–60 min, at room temperature.
6. Rinse in water, then differentiate if necessary in 1% HCl in 70% ethanol.
7. Dehydrate, clear and mount.

Elastic fibres, copper-associated protein and hepatitis B surface material (HBsAg) stain brown. The method is less sensitive for HBsAg than immunohistochemical techniques. However, of the components listed, copper-associated protein is often the most difficult to stain reliably. Natural orceins seem to be more satisfactory than synthetic ones, but are difficult or impossible to obtain. In case of difficulty, doubling the concentration of orcein and the amount of HCl may help (Hans Popper, personal communication).

Acidified potassium permanganate

To 95 ml of 0.5% potassium permanganate, add 5 ml of 3% sulphuric acid.

Box 2.1 Continued

Rhodanine stain for copper[5,6,7]

1. Bring section to distilled water.
2. Incubate in rhodanine working solution for 18 h at 37°C or 3 h at 56°C.
3. Rinse in several changes of distilled water and stain with Carazzi's haematoxylin for 1 min.
4. Rinse with distilled water and then quickly in borax solution. Rinse well in distilled water.
5. Dehydrate, clear and mount.

Copper deposits stain bright red. Bile stains green. Weakly positive stains tend to fade, but fading can be reduced by staining at the higher temperature and by using certain mounting media (e.g. Ralmount (Raymond A. Lamb), DPX or Diatex). Note the two alternative times and temperatures for the rhodanine working solutions. The staining time can be shortened further.[5]

Rhodanine stock solution

p-Dimethylaminobenzylidene rhodanine	0.2 g
Ethanol	100 ml

The working solution is prepared by diluting 3 ml of the well-shaken stock solution with 47 ml distilled water.

Borax solution

Disodium tetraborate	0.5 g
Distilled water	100 ml

Victoria blue method for copper-associated protein, elastic fibres and hepatitis B surface material[8]

1. Bring section to distilled water.
2. Treat with acidified potassium permanganate (see Gordon & Sweets' reticulin, above) for 5 min.
3. Treat with 4% aqueous sodium metabisulphite for 1 min.
4. Wash in running tap water.
5. Wash well with 70% ethanol.
6. Stain in Victoria blue solution in a Coplin jar for a minimum of 4 h, and preferably overnight.
7. Wash well with 70% ethanol. This is the differentiation step; ensure that the background of the section is clear.
8. Wash in running tap water for 1 min.
9. Stain with nuclear fast red solution for 5 min.
10. Wash in running water for 2 min.
11. Dehydrate, clear and mount.

Copper-associated protein, elastic fibres and HBsAg are stained blue on a pink background.

Victoria blue solution

Distilled water	200 ml
Dextrine	0.5 g
Victoria blue	2 g
Resorcinol	4 g

Box 2.1 Continued

Slowly warm the mixture of the above until it boils. Gradually add 25 ml of boiling 29% ferric chloride solution and boil for a further 3 min. Cool and filter through fine paper. Dry the filtrate on the filter paper to complete dryness in a 56°C oven. Dissolve the filtrate in 400 ml 70% ethanol. Finally add 4 ml concentrated HCl and 6 g phenol. The solution is best left for 2 weeks before use.

Nuclear fast red

Dissolve 0.1 g nuclear fast red in 100 ml warmed 5% aluminium sulphate. Filter when cool.

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General reading

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CHAPTER

3 The Normal Liver

Structures and components

Functional units and nomenclature

Under the low power of the light microscope, normal liver is seen to have a regular structure based on portal tracts and efferent veins. The smallest portal tracts contain portal venules, hepatic arterioles and small interlobular bile ducts. Blood from both venules and arterioles passes through the sinusoidal system to reach efferent hepatic venules. From these, the blood drains into successively larger veins to reach the inferior vena cava. Bile flows from the smallest ducts into larger ducts, to reach the small intestine by way of the common bile duct.

The functional relationship between these various structures has been the subject of much debate. The most widely used models are the classic lobule and Rappaport's acinus.[1] The lobule has an efferent venule at its centre and portal tracts at its periphery (**Fig. 3.1**). The acinus is based on a terminal portal tract, with blood passing from this, through successively less well-oxygenated parenchymal zones 1, 2 and 3, to efferent venules. It is worth emphasising that both lobules and acini are concepts rather than fixed anatomical structures. Several other models have been proposed, as well as modifications to the original lobular model.[2–4] From a pathologist's point of view, both lobular and acinar concepts have their merits in different situations. To give examples, the sinusoidal congestion of venous outflow obstruction is often more easily understood on the basis of the lobule, with maximum intensity at its centre. Bridging hepatic necrosis, however, is difficult to understand in terms of the lobule and has been explained as death of hepatocytes in acinar zones 3, the zones in which oxygen saturation is relatively low. In everyday practice it seems best to use words compatible with either model as far as possible. In this book we have therefore used the term 'periportal' to describe the part of the parenchyma lying nearest to a small portal tract, and 'perivenular' for the parenchyma near an efferent venule.

Portal tracts

Portal tracts of different size may be seen in biopsies (**see Fig. 4.2**). The smallest represent terminal tracts from which blood enters the parenchyma. Larger portal tracts contain vessels and ducts which convey blood and bile to and from the smaller tracts. Pathological processes do not necessarily affect large and small tracts to the same extent.

A typical small portal tract contains a bile duct, portal venule, hepatic arteriole and lymphatics, all embedded in connective tissue (**Fig. 3.2**). A few lymphocytes and mast cells may be seen even in normal subjects and nerve fibres can be demonstrated by appropriate staining. The exact contents are variable, however, depending in part on the angle of

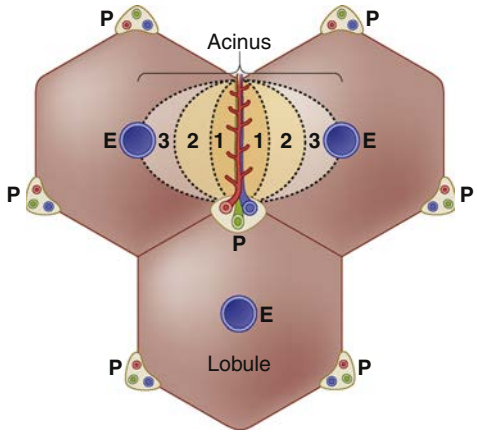

Fig. 3.1 Diagrammatic representation of a simple acinus. It is divided into zones 1, 2 and 3, with three adjacent lobules for comparison. Portal tracts (P) contain bile ducts, arterioles and venules. E, efferent vein (central vein or terminal hepatic venule).

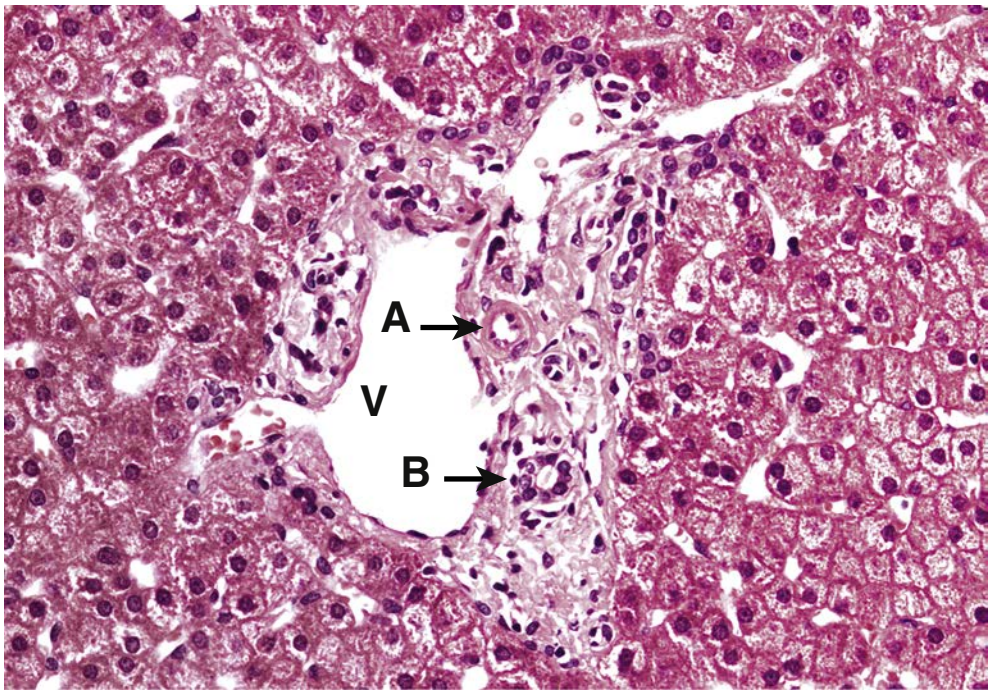

Fig. 3.2 Normal adult liver. A small portal tract contains a portal venule (V), arteriole (A) and interlobular bile duct (B). (Needle biopsy, H&E.)

sectioning. In a study of 16 needle biopsies from normal subjects,[5] 38%, 9% and 7% of tracts did not contain a portal-vein branch, hepatic arteriole or bile duct, respectively. Most, but by no means all, hepatic artery branches are accompanied by bile ducts. These observations have obvious implications for the histological diagnosis of bile duct or blood vessel loss. A confident diagnosis requires examination of several portal tracts.

Bile ducts

Near or at the margins of the small portal tracts, the bile canaliculi, formed as spaces between adjacent hepatocytes, communicate with the canals of Hering.[6–8] These are lined

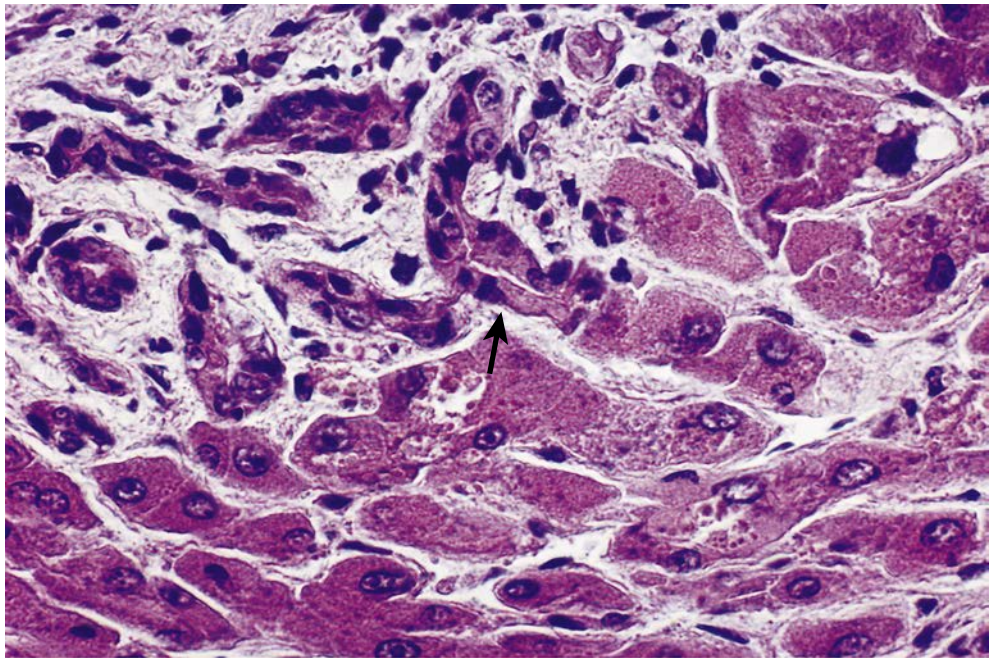

Fig. 3.3 Bile ductules and canals of Hering. These are unusually prominent in this cirrhotic liver. A liver cell plate is seen in continuity with a ductular structure (arrow). (Needle biopsy, H&E.)

partly by hepatocytes and partly by biliary epithelial cells. From the canals of Hering, bile drains into bile ductules lined entirely by biliary epithelium (**see Fig. 5.1**). Neither canals of Hering nor ductules are easily seen in normal liver, but they may become apparent in disease (**Fig. 3.3**). The exact location of the junction between the canals of Hering and bile ductules varies, the ductules sometimes having an intraparenchymal portion, seen in two-dimensional sections as apparently isolated ductules among hepatocytes. The canals of Hering and bile ductules have received much attention in recent years because they appear to be the site of a progenitor-cell compartment which becomes activated when a need for new hepatocytes and bile ducts cannot be adequately met otherwise.[8–10] Progenitor cells can be immunostained for cytokeratins CK7 and CK19, EpCAM (epithelial cell adhesion molecule) and NCAM (neural cell adhesion molecule)[11] and also stain with OV-6, an antibody used on frozen tissue to mark similar cells (oval cells) in rodents.[11,12] The response to various types of liver injury may also involve participation of hepatoblasts derived from progenitor cells.[12]

The interlobular ducts into which the ductules drain have an internal diameter of less than 100 μm and are more or less centrally located in the small portal tracts. They are lined by cuboidal or low columnar epithelium and have a basement membrane associated with diastase periodic acid–Schiff (DPAS)-positive material. Portal venules and hepatic arterioles usually lie close to these ducts but, as already noted, not all three structures are necessarily seen in a single plane of section. Positive identification of bile ducts in pathological states can be difficult, but is made easier by cytokeratin staining; ducts contain CK7 and CK19 in addition to CK8 and CK18; the latter two are also found in hepatocytes.[13]

Bile drains from the interlobular ducts into septal bile ducts having an internal diameter of more than 100 μm. Septal ducts are lined by tall columnar epithelium, with basally located nuclei. These and larger ducts towards the hepatic hilum are sometimes associated with heterotopic exocrine pancreatic tissue.[14] Around the largest intrahepatic ducts there are peribiliary glands.[14]

Hepatic sinusoids, space of Disse and extracellular matrix

Hepatic Sinusoids

The hepatic sinusoids are lined by specialised endothelial cells which form an incomplete, porous barrier allowing easy exchange of materials between blood and hepatocytes. The endothelial cells are positive for cluster differentiation markers CD4, CD13, CD14, CD16, CDw32, CD36 and CD54 and thus have a different phenotype from capillary endothelium, portal venules and terminal hepatic venules. The endothelium of portal tract arterioles and portal vein branches, periportal inlet venules and central veins binds *Ulex europaeus* lectin and stains positively with CD34 and CD31,[15] whereas the sinusoidal endothelium of most of the lobule is normally negative (**Fig. 3.4**). Positive sinusoidal staining, by contrast, is seen in hepatocellular carcinoma[16] and, in a patchy distribution, in several benign conditions.[16,17]

Within the sinusoidal lumen lie the Kupffer cells, specialised hepatic macrophages which are demonstrable with immunostain for CD68. These have irregular processes, which may straddle the sinusoidal lumen. They are more numerous near portal tracts. Activated Kupffer cells, unlike endothelial cells, are DPAS- and muramidase-positive. Phenotypically distinct lymphocytes are found both within the sinusoidal lumens and in the portal tracts.[18] Lymphocytes in the lumens include pit cells having natural killer (NK) activity.[19]

Space of Disse

The space of Disse, lying between the sinusoidal endothelium and the hepatocytes, is not conspicuous in paraffin-embedded biopsies, but may be artefactually prominent in autopsy

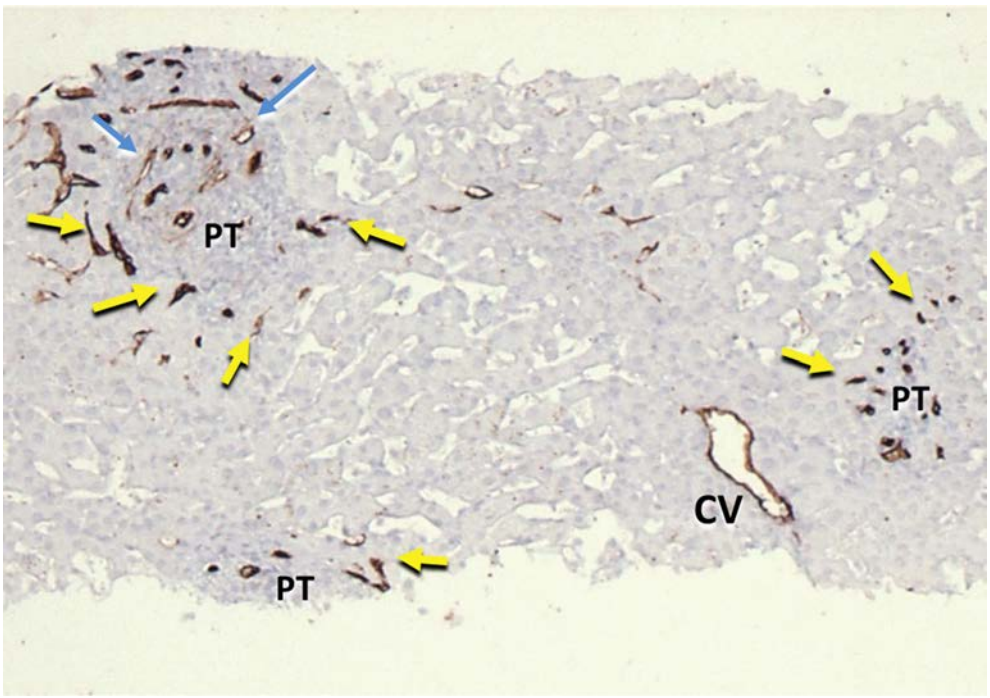

Fig. 3.4 Normal distribution of CD34 vascular endothelial staining in liver. Unlike other vascularised organs with capillaries, the hepatic sinusoids do not demonstrate positivity for CD34. Normally, only portal vein and hepatic artery branches (blue arrows), within the portal tracts (PT), inlet venules (yellow arrows) and central veins (CV) show positive staining for CD34. Most of the liver parenchyma in this biopsy shows sinusoids devoid of staining. (Needle biopsy, CD34-specific immunoperoxidase.)

material. It contains components of the extracellular matrix, nerves[20,21] and hepatic stellate cells.

Hepatic stellate cells are members of the myofibroblast family. There is international agreement that the term 'stellate cell' should be used rather than one of many synonyms in the literature[22] (see Glossary). Stellate cells are involved in fibrogenesis and in the control of sinusoidal blood flow.[23,24] They may also act as antigen-presenting cells. In childhood and adolescence, stellate cells are positive for alpha smooth-muscle actin, but thereafter become negative until activated under pathological conditions.[25] Both resting and activated stellate cells are positive for synaptophysin,[26] for vinculin after microwave pretreatment of paraffin sections[27] and for cellular retinol-binding protein-1 (CRBP-1).[28] Difficult to identify in normal liver in routine sections, stellate cells can be recognised in pathological conditions by their vacuolated cytoplasm and consequently scalloped nucleus (**see Fig. 7.8**). It is likely that the hepatic stellate cell is not the only cell type in the liver concerned with collagen synthesis.[29,30]

The extracellular matrix comprises many different components. Collagen types I and III predominate. Types IV, V, VI, VIII, XIV, XVIII and XIX are also present, together with proteoglycans and glycoproteins such as fibronectin and laminin.[31] Type III collagen is the main component of reticulin fibres in the space of Disse (**Fig. 3.5**), whereas type I is abundant in portal tracts and in the walls of efferent veins. Elastic fibres, abundant in portal tracts, are not demonstrable in sinusoidal walls in normal liver.[32]

Hepatocytes

The hepatocytes are arranged in plates separated by the sinusoidal labyrinth (**Fig. 3.6**). The layer of hepatocytes next to a small portal tract is known as the limiting plate. In adults the hepatocyte plates are one cell thick, but in any one section a few plates will appear thicker because of tangential cutting. Widespread formation of twin-cell plates indicates hyperplasia, recent or current.

Hepatocytes are polygonal cells with well-defined cell borders. Each cell contains one or more nuclei. Most cells contain one nucleus; a few contain two in normal subjects. Nucleoli are often visible, mitotic figures rare. Most of the nuclei are diploid,[33] but smaller numbers of tetraploid and even larger nuclei are found, especially in older subjects.[34] Polyploidy

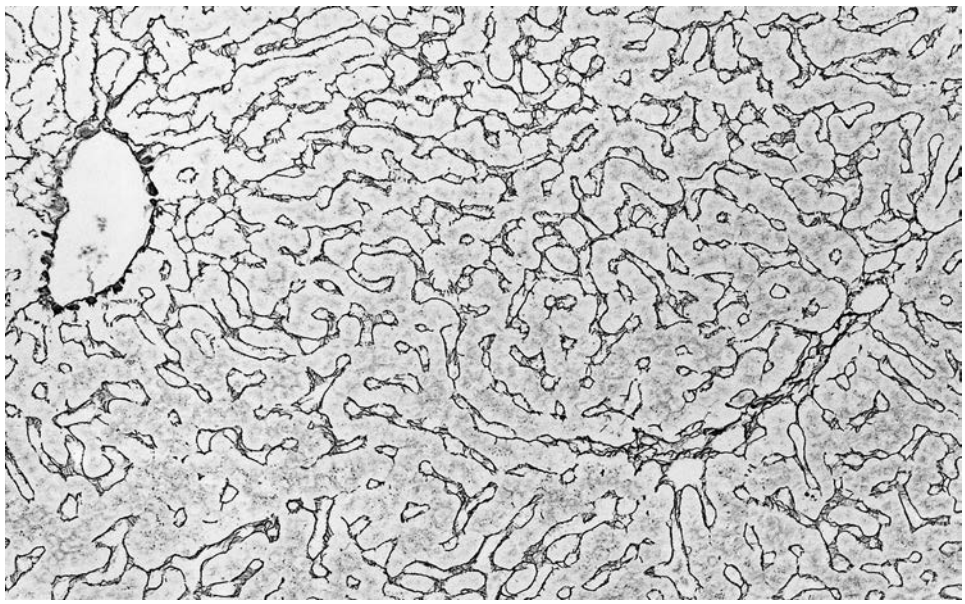

Fig. 3.5 Normal adult liver. There is a regular reticulin network between the portal tract (below right) and the efferent hepatic venule to the left. (Needle biopsy, reticulin.)

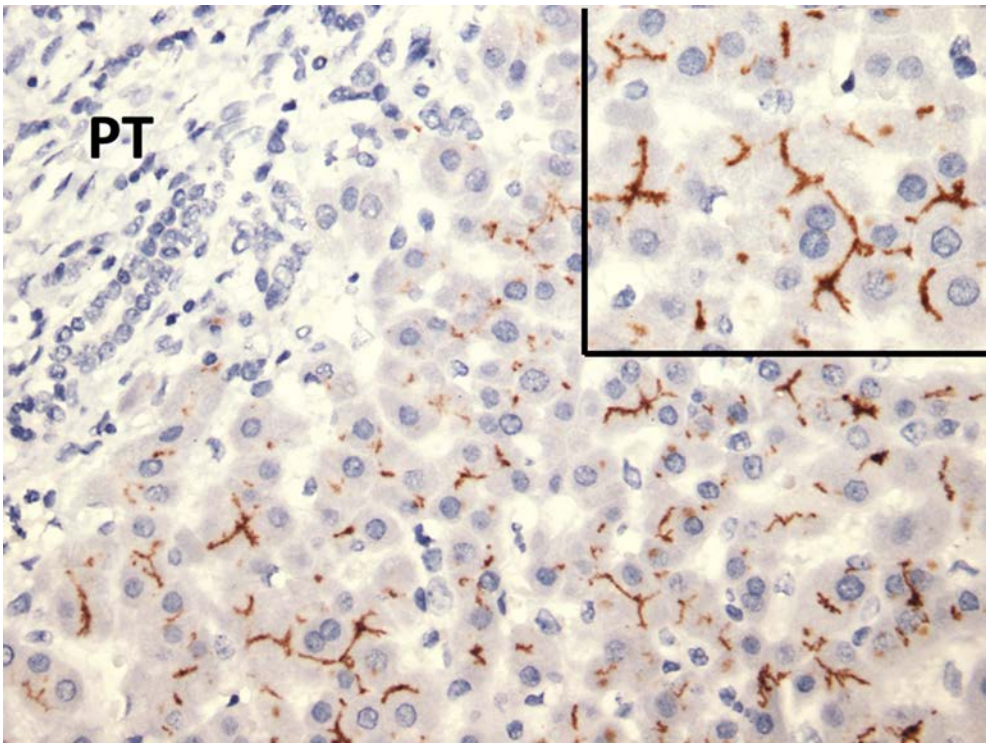

Fig. 3.6 Normal bile canaliculi. Immunostain for bile salt export pump (BSEP) demonstrates the extensive network of bile canaliculi formed by adjacent hepatocytes. INSET: Bile canaliculi are seen in branching longitudinal and cross-sectional planes. PT, portal tract. (Explant liver, specific immunoperoxidase.).

and variation in nuclear size are therefore normal characteristics of adult human liver. A few periportal nuclei may appear vacuolated because of glycogen accumulation, especially in children and adolescents.

Hepatocyte cytoplasm is normally rich in glycogen. In sections stained with haematoxylin and eosin (H&E) the cytoplasm appears granular and often pale-staining centrally, where glycogen and endoplasmic reticulum predominate. A few fat vacuoles and occasional apoptosis may be seen in the absence of obvious disease. Many different proteins can be demonstrated in or on the hepatocytes, in keeping with the liver's many metabolic functions. These include secreted proteins such as albumin and cell-surface proteins such as adhesion molecules.[35] Structural proteins include cytokeratins 8 and 18. Staining with the antibody Hep Par 1 is positive,[36] but this is not exclusive to hepatocytes.

Between the hepatocytes, their walls formed by two or three cells, are the bile canaliculi, already mentioned. They are usually too small to be readily seen by light microscopy in routine paraffin sections, but are occasionally visible as minute spaces at the biliary poles of the hepatocytes. Bile is rarely seen in normal subjects. The canalicular network can be demonstrated with a variety of immunostains, including bile salt export pump (BSEP; **Fig. 3.7**) and multidrug-resistant protein 3 (MDR3)[37,38] and polyclonal carcinoembryonic antigen (pCEA) for biliary glycoprotein on the canalicular membrane.[39] Another option is antibody to CD10 (neutral endopeptidase), which is expressed on the surface microvilli of bile canaliculi and on the apices of cholangiocytes.[40] It should be noted that physiological expression of CD10 on canaliculi develops only after 24 months of age[40] and, consequently, immunostain results will be negative in younger children and neonates.

Hepatocellular pigments

A variety of pigments may be seen in liver tissue (Table 3.1). Within the hepatocytes, aggregated near the bile canaliculi and most abundant in perivenular areas, there are fine

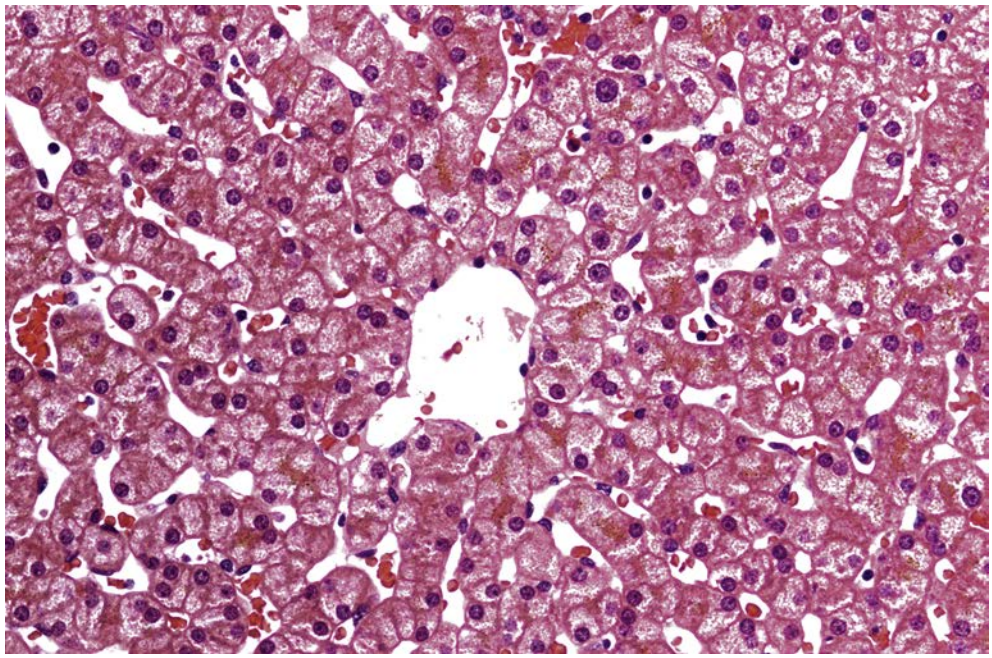

Fig. 3.7 Normal adult liver. Hepatocyte plates, for the most part one cell thick, radiate out from the terminal venule in the centre. (Wedge biopsy, H&E.)

Table 3.1 Identification of hepatocellular pigments.

	Haemosiderin	Lipofuscin	Dubin–Johnson pigment	Bile	Copper-associated protein
Distribution	Periportal	Perivenular	Perivenular, often also in Kupffer cells	Often perivenular; also in canaliculi and Kupffer cells	Periportal in chronic cholestasis
Intracellular site	Pericanalicular	Pericanalicular	Pericanalicular	Pericanalicular or diffuse	Variable
Granule size (approximate)	1 μm	1 μm often	>1 μm	Variable	≤1 μm
Colour	Golden brown, refractile	Yellow brown	Dark brown	Yellow, brown or green	Grey
Perls' stain for iron	+	–	–	–	–
Diastase–PAS stain	–	Variable	Variable	Variable	Often +
Long Ziehl–Neelsen stain	–	+	Often +	–	–
Orcein, Victoria blue stain	–	–	–	–	+

PAS, periodic acid–Schiff.

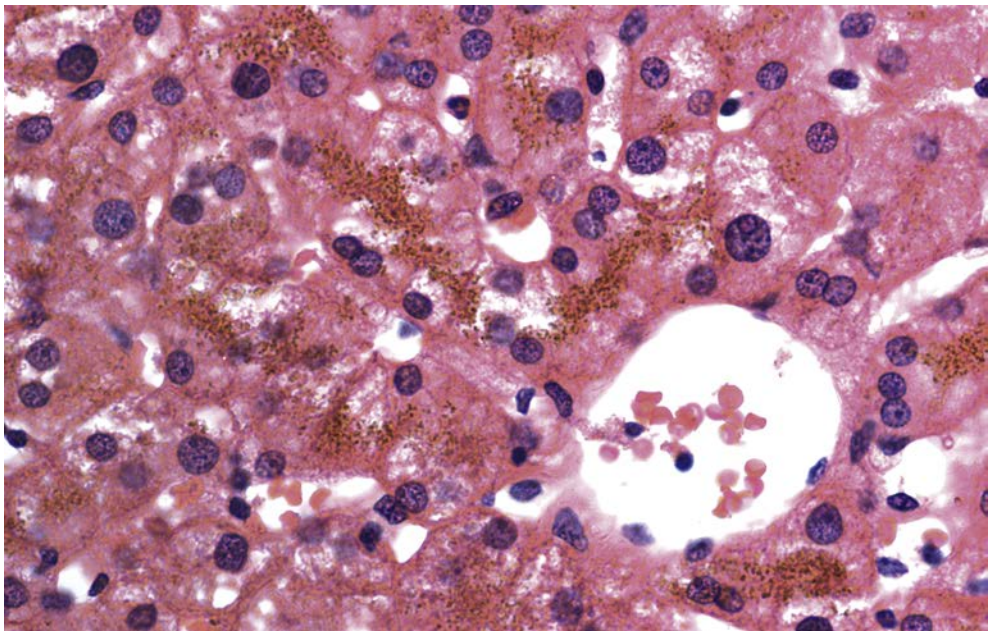

Fig. 3.8 Lipofuscin pigment. In this normal liver from an adult there are prominent brown lipofuscin granules at the biliary poles of the hepatocytes. (Wedge biopsy, H&E.)

yellow-brown granules of lipofuscin pigment (**Fig. 3.8**). Lipofuscin is a normal constituent of adult liver, increasing in amount with age but also sometimes found in children. The granules represent lysosomes containing materials which cannot be further degraded. The amount of the pigment varies greatly in normal liver, making assessment of an increase or decrease in disease subject to error in the absence of well-controlled morphometric data. Lipofuscin also varies in its staining properties according to its constituents and age. It is acid-fast, has reducing properties and stains variably with DPAS. Perls' stain for iron is negative.

Large amounts of lipofuscin are difficult to distinguish from Dubin–Johnson pigment by light microscopy alone, but the latter is usually coarser and darker (**see Fig. 13.21**). Intracellular bile can be distinguished from lipofuscin by its bright green staining with Van Gieson's method (**see Fig. 4.10**) and by the almost invariable presence of bile thrombi in canaliculi. An exception to this is liver following transplantation, in which diffuse intracellular bile is common in the absence of bile thrombi.

Normal liver is negative for stainable iron. All but very small amounts should be further investigated by appropriate biochemical and genetic methods. This is because it is important to identify patients with the common and treatable condition of hereditary haemochromatosis (**Ch. 14**).

Copper-associated protein is seen in high copper states as grey-brown or red intracytoplasmic granules, usually in a periportal location. It can be stained with orcein, Victoria blue and DPAS.

Normal appearances in childhood

Haematopoiesis is active during the fetal period (**Fig. 3.9**) and continues until a few weeks after birth. Haemopoietic cells are present in portal tracts and sinusoids (**Fig. 3.10**). Hepatocyte plates are mainly two cells thick until the age of 5 or 6 years, when the adult pattern of single-cell plates is established. Hepatocytes and their nuclei vary little in size. Glycogen vacuolation of nuclei is common until adolescence. Lipofuscin pigment is absent or scanty in the first two decades of life.

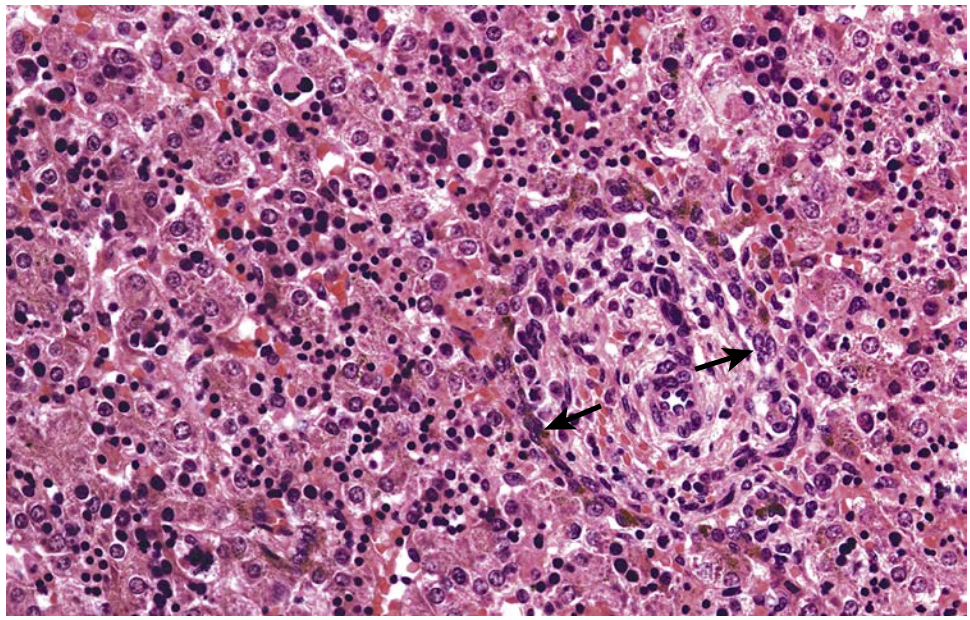

Fig. 3.9 Liver of fetus at 19 weeks' gestation. Many haemopoietic cells are seen in sinusoids and in the immature portal tract. A ductal plate at the margin of the tract (arrows) indicates bile-duct formation. (Postmortem liver, H&E.)

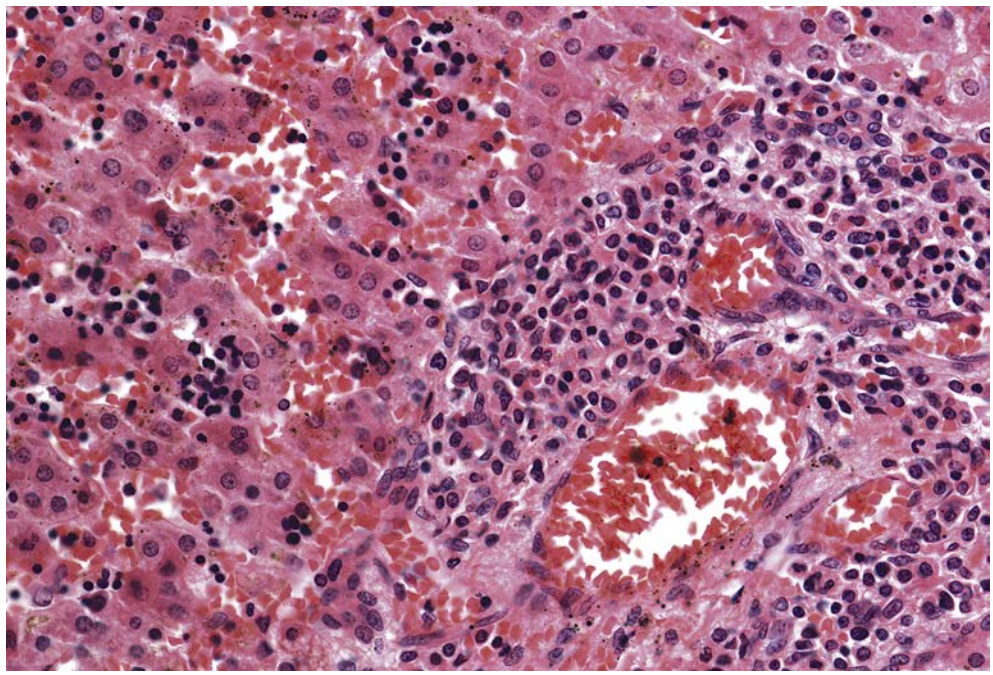

Fig. 3.10 Normal liver in a neonate. Abundant haemopoietic cells are seen in the portal tract and in the sinusoids. (Postmortem liver, H&E.)

Ageing

The size of hepatocytes and their nuclei becomes more variable with increasing age, most notable in perivenular regions (Fig. 3.11). This variation is due to greater numbers of polyploid cells,[34] with large nuclear and cell volumes. Lipofuscin pigment in hepatocytes is often abundant, especially around terminal hepatic venules (Fig. 3.8). Portal connective

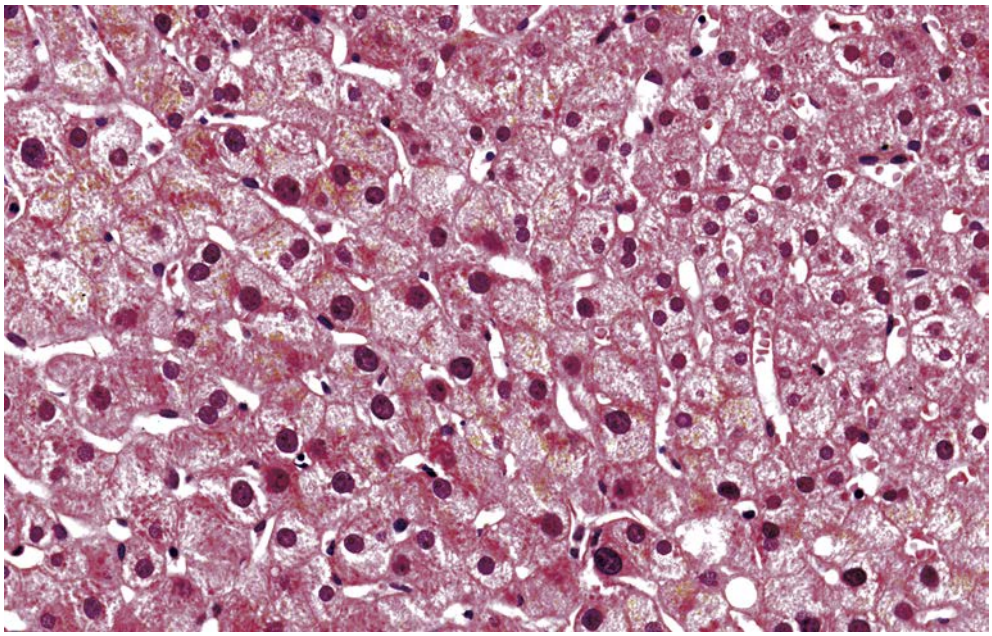

Fig. 3.11 Liver in an elderly person. Hepatocyte nuclei vary considerably in size. (Needle biopsy, H&E.)

tissue becomes denser, and arteries may be thick-walled, even in normotensive subjects.[41] Pseudocapillarisation of the sinusoidal lining with loss of permeability may have important consequences for lipid metabolism and vascular disease.[42]

Biopsy of the normal liver

Percutaneous liver biopsies are necessarily taken though the liver capsule, which may be seen at one end of the core or as a separate piece. It sometimes contains vessels and bile ducts, but can be distinguished from a pathological septum by the density and maturity of the connective tissue. Deeper in the core, pathological septa must also be distinguished from longitudinally cut portal tracts (**Fig. 3.12**). The length and width of the liver core are often critical for diagnosis, as discussed under the heading of grading and staging in **Chapter 9**. Short pieces or slender cores taken with narrow needles may be inadequate for the diagnosis of unevenly distributed, non-neoplastic lesions.

Other organs and tissues, especially skin, pleura and intercostal muscle, are sometimes included in the specimen. Close apposition to the liver core of fibrous tissue or of tumour does not necessarily reflect hepatic fibrosis or tumour within the liver.

Transjugular biopsy is now often used and usually provides ample-sized specimens, sometimes including longitudinally cut portions of efferent vein walls (**see Fig. 1.4**).

Surgical biopsies taken from the inferior margin of the liver are in the form of wedges covered on two aspects by capsule. The structure of the immediately subcapsular zone differs somewhat from the deeper tissue (**Fig. 3.13**), but there is good correlation between the volume fraction of non-parenchymal components in subcapsular and deeper zones.[43] Appearances mimicking cirrhosis do not usually extend for more than 2 mm into the liver, and confusion is unlikely except with very small superficial samples.

In surgical biopsies taken some time after the beginning of an operation, neutrophil leukocytes accumulate under the capsule and in portal tracts, around terminal venules and focally within the parenchyma (**Fig. 3.14**). Here, there is focal loss of hepatocytes. Similar

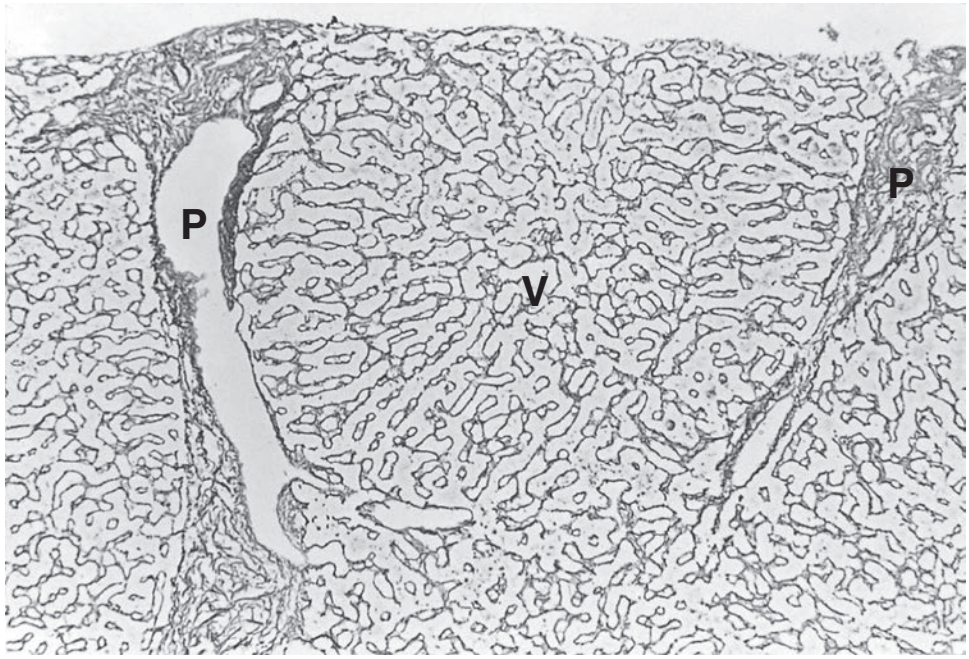

Fig. 3.12 Normal adult liver. Two normal portal tracts (P), cut longitudinally, mimic septa. Between them is an efferent hepatic venule (V). (Needle biopsy, reticulin.)

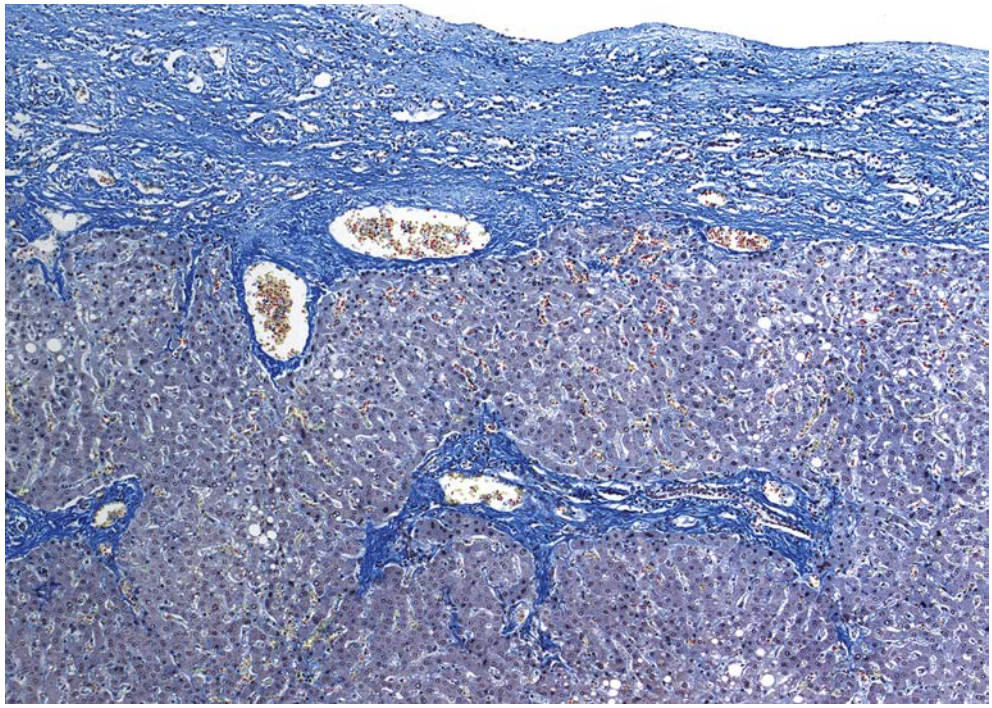

Fig. 3.13 Normal adult liver. The capsule is thick and portal tracts are prominent. (Postmortem liver, trichrome.)

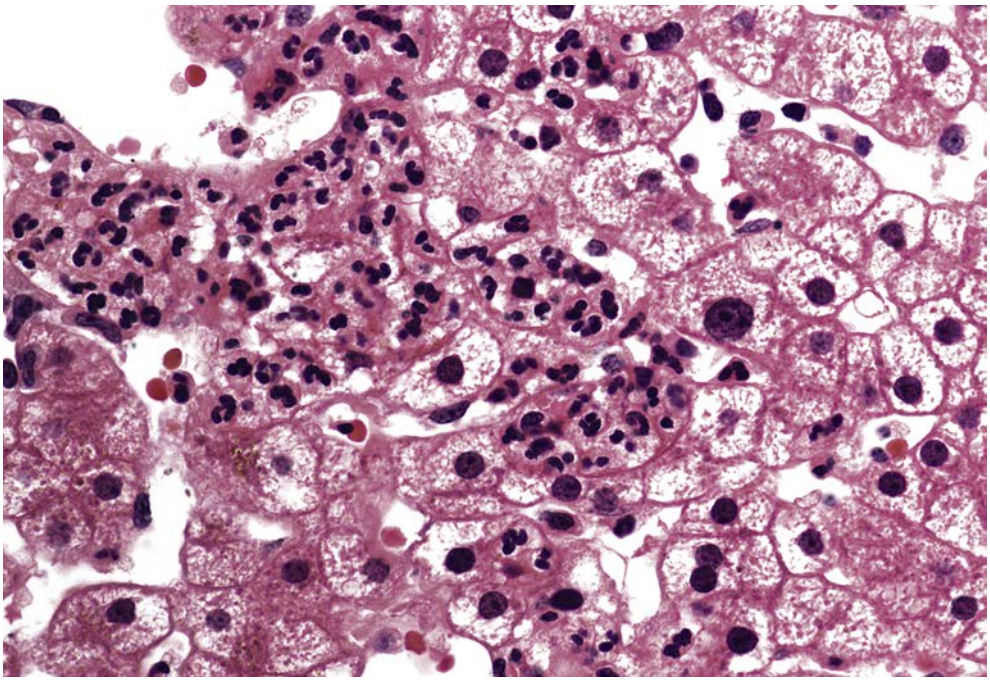

Fig. 3.14 Operative wedge biopsy. Clumps of neutrophils mark sites of hepatocellular necrosis, resulting from the procedure. Part of an efferent venule is seen (top left). (Wedge biopsy, H&E.)

parenchymal changes have been reported after heavy sedation without full anaesthesia.[44] They are also found in patients infected with cytomegalovirus (**Ch. 15**).

Liver biopsies obtained to evaluate abnormal serum liver function tests or to answer other clinical questions may, infrequently, be close to normal ('near-normal' or 'almost-normal' liver biopsy).[45] Such biopsies may show sparse portal inflammation (without interface hepatitis), rare lipid in hepatocytes or other non-specific features, but otherwise demonstrate no definitive diagnostic pathology. Such cases clearly warrant close microscopic scrutiny and also benefit from exclusion of a short list of 'pertinent negatives' (**Box 3.1**). Some patients later progress to specific disorders such as autoimmune hepatitis or primary biliary cholangitis.[45]

Box 3.1 Checklist of pertinent negatives in the examination of liver biopsies*

- Steatosis (macrovesicular and/or microvesicular)
- Cholestasis
- Hemosiderosis
- Ground-glass inclusions
- Alpha-1-antitrypsin globules in periportal hepatocytes (on routine H&E and diastase–PAS stains)
- Native bile ducts are present and preserved

**Prior to completing the microscopic examination of liver biopsy specimens (especially 'near-normal' liver biopsies) this checklist should be reviewed so as to avoid missing important, but not necessarily conspicuous lesions. H&E, haematoxylin and eosin; PAS, periodic acid–Schiff.*

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CHAPTER

4 Assessment and Differential Diagnosis of Pathological Features

Initial examination and reporting

Naked-eye examination and description of biopsy specimens

Although naked-eye examination and description are of limited diagnostic value, they reduce the possibility of specimen identification error. The pathologist should make sure that the whole specimen has been adequately sectioned by comparing the size of the sectioned and stained tissue with the measurement recorded on macroscopic examination. Naked-eye examination also helps in the selection of suitable areas for electron microscopy. The contour and colour of needle biopsy specimens in the fixative container, in the paraffin block or on the glass slide itself may provide some preliminary diagnostic impressions, barring any technical artefacts imposed by unusual specimen handling or staining. Normal liver gives rise to cylinders of even colour and thickness, which do not fragment easily. By this standard, needle biopsies can usually be categorised as one of the following three types, based on their contours (**Fig. 4.1**): (1) *normal contour* (suggesting relatively intact architecture without advanced fibrosis, although significant pathology such as hepatitis, cholestasis or other findings may nonetheless be present); (2) *irregular contour* (suggesting the presence of chronic disease, with focal regions of narrowing due to substantial fibrosis or cirrhosis); and (3) *fragmented biopsy* (consistent with cirrhosis, primary hepatocellular carcinoma or metastatic tumour). Such impressions obviously require further confirmation on microscopy. Cholestasis imparts a green colour, whereas fatty liver is pale brown or yellow and may float in the fixative. In cholesterol ester storage disease and Wolman's disease the specimen is bright orange; this should warn the pathologist of the need to keep some tissue for frozen sectioning and electron microscopy. A black or very dark brown colour is characteristic of the Dubin–Johnson syndrome. Metastatic tumour, like fibrous tissue, is often white. Congested liver is deep red in colour.

Routine microscopy

Routine microscopy of liver biopsies should include systematic assessment of overall structure, portal tracts and their contents, terminal hepatic venules, hepatocytes and sinusoidal cells. Some pathologists use a pro forma or checklist in order to avoid omitting relevant data.[1] It is often helpful to make certain that several relatively common lesions have not been missed, such as cholestasis, steatosis, hemosiderosis, ground-glass hepatocellular inclusions,

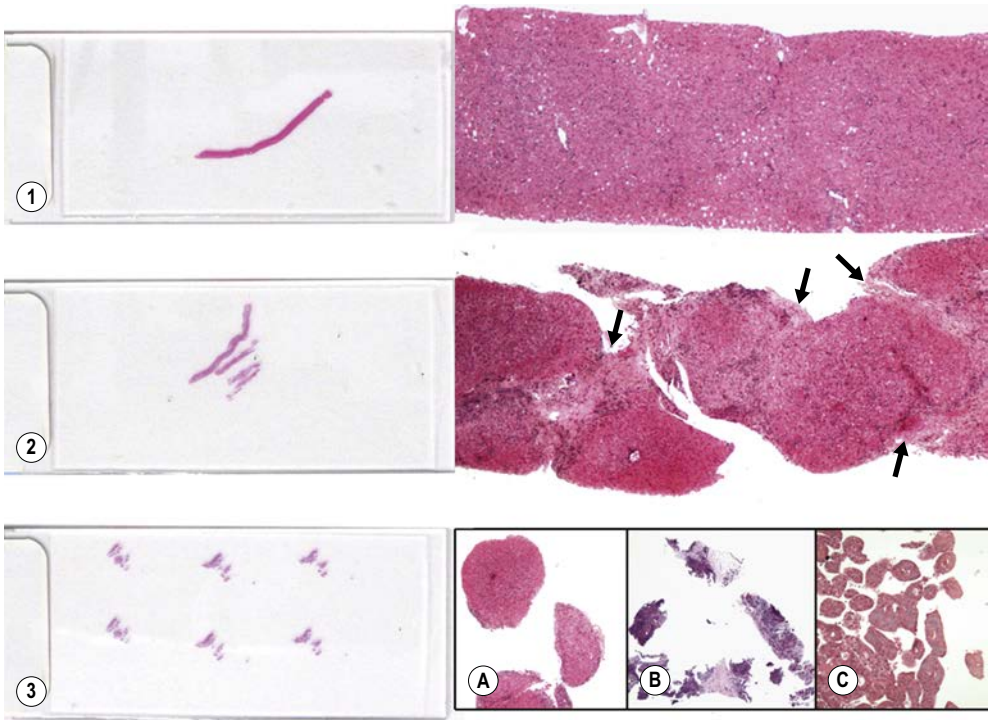


Fig. 4.1 Variations in needle liver biopsy specimens. The contours of the needle biopsy cores on the glass slide may offer some preliminary diagnostic impressions, usually falling into one of the following three categories: (1) normal contour (suggesting relatively preserved architecture without extensive fibrosis, although chronic hepatitis, steatosis or other disease may be present); (2) irregular contour, where fibrosis or cirrhosis has resulted in focal narrowing (fibrotic portal tracts shown at arrows); and (3) fragmentation, usually due to one of three conditions: cirrhosis (**A**), metastatic tumour (**B**) or primary hepatocellular carcinoma (**C**). (Needle biopsies, H&E.)

periportal alpha-1-antitrypsin globules and bile duct loss. The pathologist should bear in mind that some liver biopsies show very few changes that are specifically diagnostic and may result in a diagnosis of 'non-specific changes" or 'near-normal liver'. The diagnosis in some of these cases only later comes to light and is a recognized condition such as autoimmune hepatitis, drug-induced liver injury or a fatty liver-related lesion.[1b]

The following sections are intended to help in the evaluation of pathological changes. Most of the information is also found in other parts of the book, under individual diseases. There is inevitably some repetition, because many of the listed features are found in combination. The final part of the chapter contains guidance on the differential diagnosis of a number of specific pathological findings.

Basic patterns of injury

Structural changes, collapse and fibrosis

Minor structural changes are difficult to assess in sections stained with haematoxylin and eosin (H&E), and may indeed be missed altogether. Examination of a connective tissue preparation is therefore often important. Normal liver tissue shows a hierarchy of ramifying portal tracts of varied sizes which are present in needle and wedge biopsy samples (**Fig. 4.2**). The subdivisions of these portal tracts parallel the hierarchy of hepatic artery and

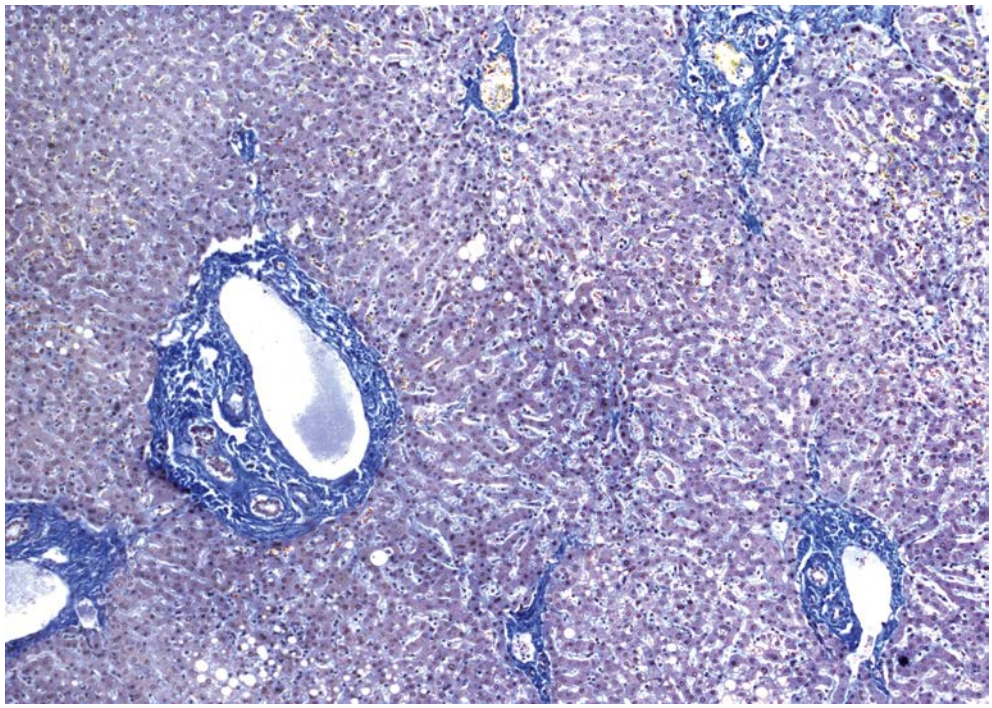


Fig. 4.2 Portal tract size variations. Biopsies contain portal tracts ranging in size from larger conducting tracts (left) to the small terminal tracts (right top and bottom) from which blood enters the parenchyma. (Wedge biopsy, chromotrope–aniline blue.)

portal vein branches and bile ducts as they distribute throughout the liver and can thereby be roughly subdivided into segmental, area, conducting (septal) and terminal portal tract units (**see Fig. 5.1**). For detection of the most minor abnormalities an uncounterstained silver impregnation for reticulin is generally best, although pericellular fibrosis is most easily detected in sections stained for collagen.

Using these methods, an impression may be gained that, although portal tracts and terminal venules are normally related to each other, the portal tracts are enlarged and perhaps even linked by fibrous septa. This is consistent with mild chronic viral hepatitis or with one of the conditions in which portal changes typically predominate; these include biliary tract disease, haemochromatosis, congenital hepatic fibrosis and schistosomiasis. If, by contrast, the reticulin framework of the parenchyma is distorted, lesions characterised by lobular damage should be considered. These include acute and chronic hepatitis as well as forms of biliary disease in which there is also hepatocellular damage, notably primary biliary cirrhosis. Venous congestion leads to regular condensation of perivenular reticulin.

Recent collapse and fibrosis are sometimes difficult to distinguish, even with the help of good collagen stains. A stain for elastic tissue can help to resolve this problem because the presence of elastic fibres outside the portal tracts is an indication of long-standing disease. Collagen stains are helpful for the recognition of blocked veins, for example, in necrotic areas, alcoholic liver disease, venous outflow obstruction and epithelioid haemangioendothelioma. Collagen staining is important for the detection of pericellular fibrosis, as already indicated, and should therefore be used whenever there is substantial steatosis or a suspicion of steatohepatitis.

The histological diagnosis of cirrhosis is fully discussed in **Chapter 10**. Once cirrhosis has developed, the pattern of fibrosis is one of the features that may help to determine its cause. In primary or secondary biliary cirrhosis, for example, fibrosis expanding and linking the portal tracts is a more important early factor in pathogenesis than hepatocellular regeneration; this is reflected in the morphological picture of broad perilobular septa surrounding irregularly shaped islands of parenchyma (**see Fig. 5.11**). In hereditary haemochromatosis and chronic venous outflow obstruction the impression is also of fibrosis rather than regeneration as the principal pathogenetic factor. In these diseases with a long

precirrhotic phase of fibrosis, transected parenchymal peninsulas may be mistaken for true regenerative nodules. This is particularly common just deep to the liver capsule. Isolated subcapsular nodules in an otherwise not nodular biopsy should therefore be interpreted with caution.

Hepatocellular damage

There is a broad histological spectrum of possible hepatocellular damage, ranging from subtle changes affecting the appearance of the cytoplasm or specific organelles to obvious hepatocyte ballooning, apoptosis or necrosis. Normal hepatocytes are polygonal in shape, with abundant pale-staining granular cytoplasm rich in glycogen. An occasional **apoptotic body** (acidophil body) may be seen in normal liver. When present, apoptotic bodies are typically seen within sinusoidal spaces following extrusion from liver-cell plates (**Fig. 4.3**). These ovoid bodies are highly eosinophilic and sometimes require through-focusing on microscopic examination because their thickness is not confocal with the surrounding tissue. In cholestasis from any cause, and in donor livers shortly after transplantation, there is often an increase in the number of apoptotic bodies as well as in mitotic figures in hepatocytes. Abundant apoptotic bodies are found in acute hepatitis from any cause.[2] They were first described by Councilman

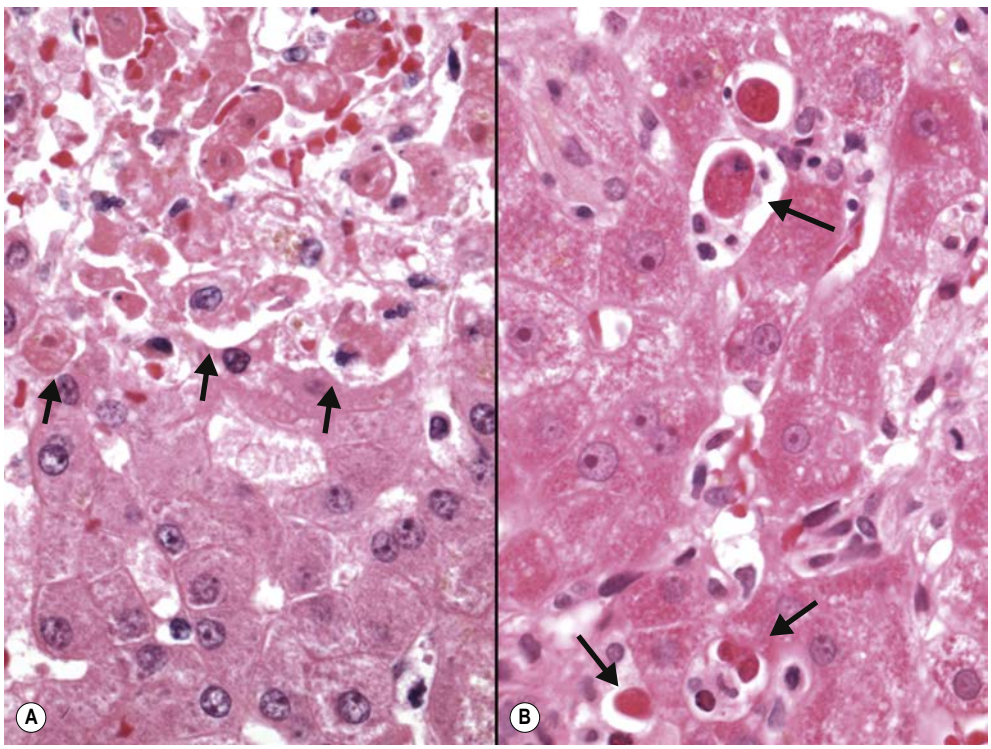


Fig. 4.3 Coagulative necrosis vs apoptosis. A: The hepatocytes above the arrows have undergone coagulative necrosis and are sharply delimited from the viable hepatocytes below. Necrosis was initiated by ischaemia due to hepatic artery thrombosis after liver transplantation. The necrotic hepatocytes show hypereosinophilia, nuclear pyknosis and discohesion. (Explant liver, H&E.) **B:** Multiple apoptotic bodies of different sizes are seen within sinusoids (arrows). The accentuated hepatocellular apoptosis was due to early recurrence of hepatitis C virus infection 2 months after transplantation. (Allograft needle biopsy, H&E.)

Table 4.1 Differential diagnosis of ground-glass hepatocytes

Condition	Staining method(s)
Chronic hepatitis B	Orcein, Victoria blue
	Immunostain for HBsAg
Medication (e.g. barbiturate)	–
Cyanamide alcohol aversion therapy	Diastase–PAS
Lafora's disease (myoclonus epilepsy)	PAS, colloidal iron
Type IV glycogenosis	PAS
Transplant recipients	PAS

HBsAg, Hepatitis B surface antigen; PAS, periodic acid–Schiff.

in yellow fever, so that the term 'Councilman body' should, strictly speaking, be confined to that disease (**see Ch. 6**).

Hepatocytes may demonstrate cytoplasmic **'ground-glass' change** in a variety of conditions[3] (**Table 4.1**). The affected liver cells have a pale pink homogeneous appearance resembling frosted glass (**Fig. 4.4**). The change may involve all or a portion of the hepatocyte cytoplasm or may be in the form of a rounded or crescentic inclusion, sometimes with a surrounding artefactual empty white space. A common example is the hepatitis B surface antigen-containing ground-glass inclusion seen in individuals with chronic hepatitis B (**Fig. 4.4A, and see Fig. 9.13**). Such inclusions are scattered randomly through the lobular parenchyma but sometimes are numerous. Use of certain medications (e.g. barbiturates) and occasionally hepatocellular cholestasis[4] can result in similar appearances, but confined to perivenular hepatocytes (**Figs 4.4B,D, and see Fig. 8.1**), where it is referred to as 'pseudo-ground-glass' change. Recipients of transplants (liver, cardiac, bone marrow) may also show ground-glass-like inclusions containing an abnormal type of glycogen[5–7] (**Fig. 4.4C**). These have a predilection for periportal hepatocytes, as do the ground-glass inclusions of Lafora's disease (myoclonus epilepsy). Assessment of the clinical setting together with the staining methods shown in **Table 4.1** usually clarifies the cause of the ground-glass change.

Moderate **hepatocyte swelling** is sometimes due to adaptive hyperplasia of smooth endoplasmic reticulum in response to drugs or, occasionally, is due to cholestasis. Perivenular hepatocyte swelling may be seen in allograft biopsies soon after liver transplantation due to preservation injury of the donor liver (**see Fig. 16.2**). More severe swelling with rounding of the cell outlines is a feature of cell damage (**Fig. 4.5A**). It may accompany canalicular cholestasis (**see Figs 5.2 and 16.9**), but is most characteristically found in various forms of hepatitis (**Fig. 4.5A and see Fig. 6.2**) where it is recognised by disruption of the liver-cell plates and by accompanying inflammatory cell infiltration. The liver-cell swelling seen in viral, drug and autoimmune hepatitis differs from that seen in **hepatocellular ballooning** of steatohepatitis where the liver cells have a clarified appearance and wisp-like strands of rarefied cytoplasm, sometimes with Mallory–Denk bodies (**Fig. 4.5B and see Fig. 7.8**). The term *hepatocyte ballooning* therefore has taken on a special significance when examining liver biopsies for evidence of steatohepatitis, as is further discussed in **Chapter 7**. In microvesicular steatosis, the cytoplasm of hepatocytes is expanded by minute fat droplets which are sometimes too small to resolve by routine microscopy. The frequent presence of larger fat vacuoles and the clinical context should help to make the diagnosis. Another type of hepatocyte swelling is seen in **feathery degeneration** (**Fig. 4.5C**), where intracellular cholestasis with retention of bile and bile salts results in mild hepatocyte enlargement and pale, rarefied and reticular, often vacuolated cytoplasm. Feathery degeneration is most often seen in association with large bile-duct obstruction.

Death of individual hepatocytes or small groups of these cells is loosely called **focal necrosis** (**Fig. 4.6**), although the mechanism may in fact be apoptosis, or even a combination of both (*necroapoptosis*[8]). The distinction cannot always be made easily by routine microscopy unless apoptotic bodies are seen. Focal necrosis is associated with accumulation of inflammatory cells of various types, including macrophages. **Spotty necrosis** (**Fig. 4.6**) is a term used for the same lesion in the context of acute hepatitis.

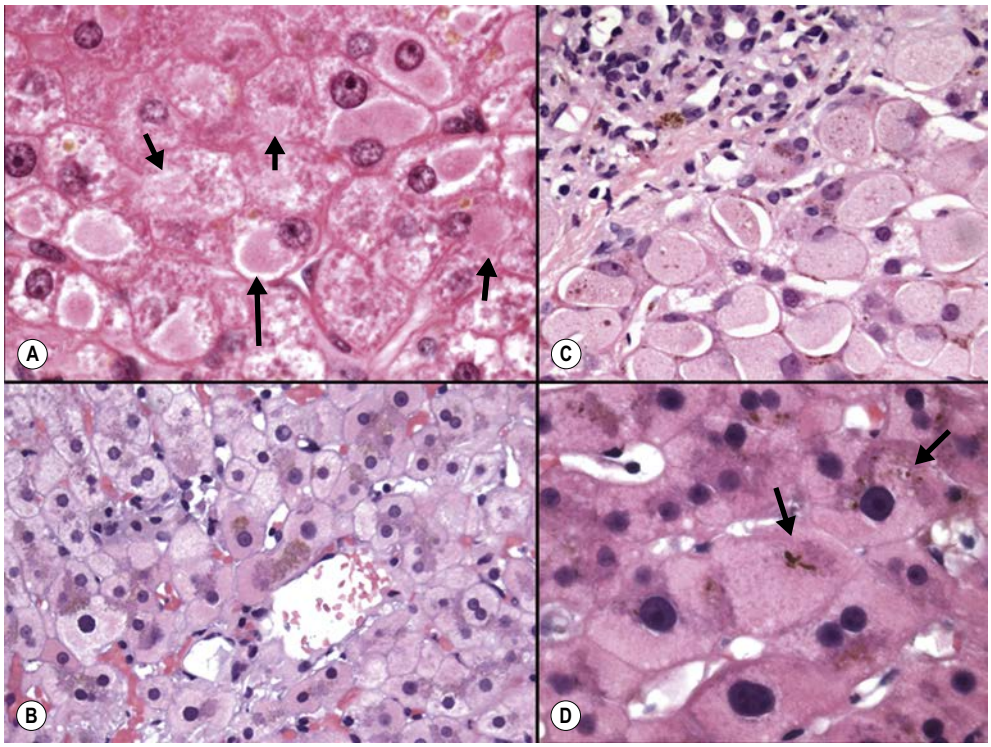


Fig. 4.4 Ground-glass and ground-glass-like hepatocytes. A: Numerous ground-glass cytoplasmic inclusions are seen within hepatocytes (large arrows) in this case of chronic hepatitis B. The inclusions represent hepatitis B virus surface antigen. Some inclusions are separated from the hepatocyte cell membrane by an artefactual empty or white halo. Even small inclusions show distinctive pale pink homogeneity (arrows). **B:** Hepatocytes around the terminal venule show pseudo-ground-glass change (induction of smooth endoplasmic reticulum) due to medication. Compare with the normal granular-appearing hepatocytes in the upper left-hand field. **C:** Liver biopsy from a bone marrow transplant recipient. There are also numerous periportal ground-glass-like inclusions. The inclusions resemble glycogen, and are thought to be a result of the many medications used in the posttransplantation clinical setting. **D:** Portions of the cytoplasm of the several hepatocytes at centre show pseudo-ground-glass change due to intracellular cholestasis (arrows). (Needle biopsies, H&E.)

Focal necrosis is a common finding which does not in itself indicate primary disease of the liver because it is often part of a non-specific reaction to disease elsewhere in the body. While degenerating hepatocytes or cell fragments are sometimes seen within the focal inflammatory infiltrate, the inflammatory reaction is usually more obvious than the necrosis, and the latter is assumed to have taken place because of a gap in a liver-cell plate (liver-cell 'dropout').

Hepatocyte death by **coagulative necrosis** (Fig. 4.3) is usually clear from its perivenular location and involvement of a contiguous group of hepatocytes in the zone of diminished perfusion. Necrotic hepatocytes show distinctive cytoplasmic eosinophilia, abnormal sizes and contours, and nuclear pyknosis and karyorrhexis. Perivenular (centrilobular, acinar zones 3) coagulative necrosis is usually seen following hypotensive or septic shock, or after hypoperfusion due to left ventricular failure or hepatic artery thrombosis. If several days have elapsed since the episode(s) of liver hypoperfusion, there sometimes is a reactive sinusoidal neutrophil infiltrate adjacent to the necrotic hepatocytes, particularly if the patient has been maintained on pressor agents.[9]

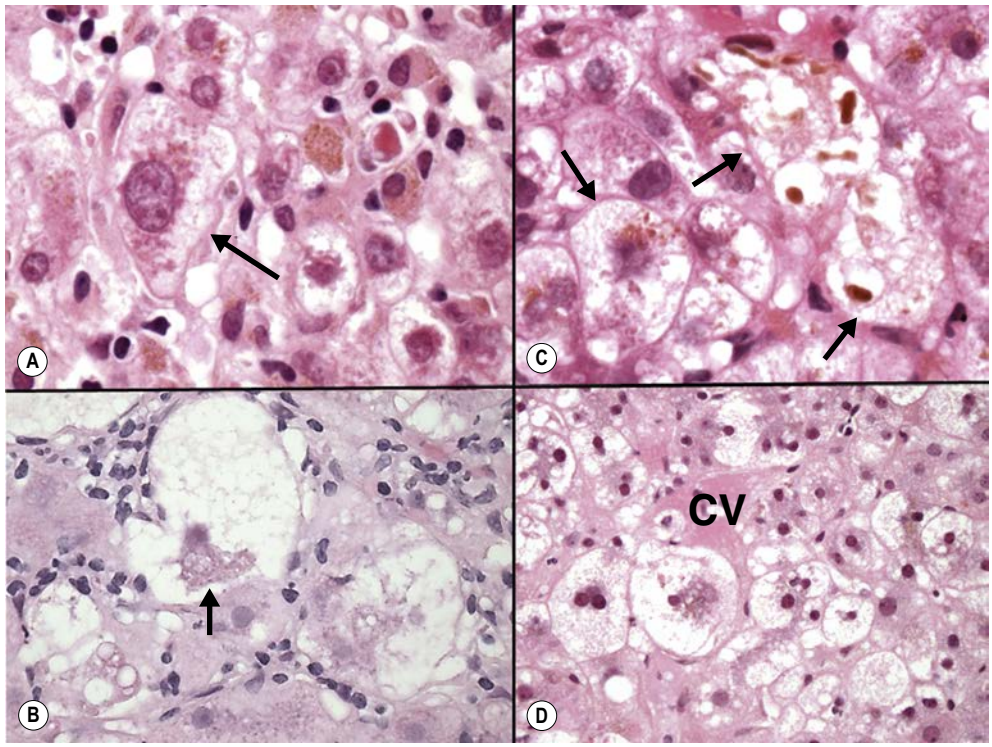


Fig. 4.5 Hepatocyte swelling and ballooning. A: A swollen and enlarged hepatocyte (arrow) is seen near lymphocytes and ceroid-laden Kupffer cells in this case of acute hepatitis. Compare the swollen liver cell to the more normal glycogenated hepatocytes at lower right. **B:** Hepatocyte ballooning in this case of non-alcoholic steatohepatitis (NASH) shows distinctive cytoplasmic rarefaction and wisp-like strands of cytoplasm. One affected hepatocyte also contains clumped eosinophilic Mallory–Denk body material (arrow). **C:** Feathery degeneration with retention of bile salts and visible bile is seen in these ballooned hepatocytes (arrows). **D:** Liver allograft biopsy obtained 1 week after transplantation because of abnormal serum liver tests. Hepatocytes around the central vein (CV) are swollen because of preservation injury. (Needle biopsies, H&E.)

Confluent necrosis

Confluent necrosis (**see Fig. 8.4**) refers to substantial areas of liver-cell death. The most common cause of this type of necrosis in biopsy material is hepatitis, whether viral, drug-related or autoimmune, in which case the necrosis is accompanied by an inflammatory reaction. Confluent necrosis with little or no inflammation is seen in hypoperfusion of the hepatic parenchyma (Fig. 4.6), as in shock or left ventricular failure, and in heatstroke (**see Fig. 12.2**). Paracetamol (acetaminophen) poisoning produces a similar lesion (**see Fig. 8.4**). In all the aforementioned examples the necrosis is typically perivenular. A predilection for mid-zonal (acinar zones 2) necrosis is seen with yellow fever (**see Fig. 6.3**) and dengue virus infections. Some poisons, including ferrous sulphate and phosphorus,[10] typically cause periportal (zone 1) necrosis. Haphazardly distributed areas of necrosis are found in disseminated herpesvirus infections (e.g. herpes simplex, varicella; **see Fig. 15.4**) and in mycobacterial diseases. Tumour necrosis may be so extensive that no recognisable tumour tissue is present in the section; in such cases the reticulin pattern may help to establish a diagnosis.

If severe and extensive, confluent necrosis may form bridges linking vascular structures and is referred to as **bridging necrosis**. Linking of portal tracts to each other is common in conditions in which portal tracts are widened, for example, by chronic hepatitis or biliary

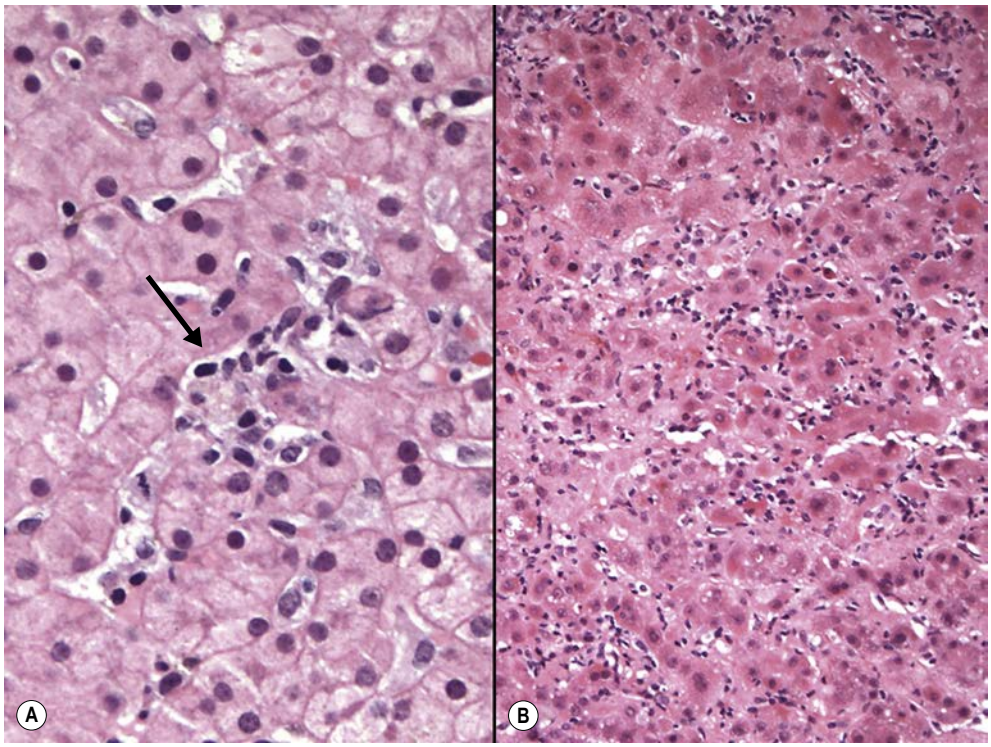


Fig. 4.6 Focal necrosis vs spotty necrosis. A: Focal necrosis (at arrow) is seen in this otherwise quiescent lobular parenchyma. The liver-cell plates are interrupted by a collection of lymphocytes and Kupffer cells where there appears to be 'liver-cell dropout'. **B:** This case of acute viral hepatitis shows 'spotty necrosis' with numerous necro-inflammatory foci throughout the lobule. (Needle biopsies, H&E.)

tract disease. Linking of perivenular areas to each other is found in some examples of parenchymal hypoperfusion and venous outflow obstruction (**Fig. 4.6**).

Bridging hepatic necrosis linking terminal hepatic venules (centrilobular veins) to portal tracts (**Figs 4.7 and 4.8**) deserves specific notation by the pathologist because of its potential association with more severe disease.[11] Central-to-portal bridging necrosis is a fairly common feature of acute hepatitis of viral type; in such cases the bridges show inflammation, loss of hepatocytes and reticulin condensation, without significant fibrosis or elastic fibres. It is also seen in exacerbations of chronic hepatitis. Old bridges contain elastic fibres as well as collagen fibres. Such bridging fibrosis is an important component of the more severe examples of both chronic viral and autoimmune hepatitis. Contraction of collagen-rich bridges may produce rapid and severe distortion of the normal hepatic microstructure, with correspondingly rapid progression to cirrhosis.

Panlobular (panacinar) and multilobular (multiacinar) necrosis (**see Fig. 6.11**) are terms used to describe confluent necrosis involving entire single lobules or several adjacent lobules, respectively. They are further discussed in **Chapter 6**. **Massive hepatic necrosis** describes loss of virtually all hepatic parenchyma and is characteristically seen in *acute liver failure (ALF)* of viral, drug, autoimmune or unknown causation.[12] Histologically there is widespread hepatocyte loss with collapse of reticulin accompanied by outgrowth of periportal bile ductular structures (neocholangioles) derived from activated hepatic progenitor cells. Such livers grossly are reduced in size and show capsular wrinkling due to loss of subcapsular parenchyma. The term **submassive necrosis** is used in certain cases which present clinically as ALF to describe severe loss of liver parenchyma (as in massive hepatic necrosis) but accompanied by foci of regenerative hyperplasia and nodules that are visible on both gross and histological examination (**Fig. 4.9**). The presence of regenerative nodules and, depending on the individual case, evidence of early fibrosis are consistent with a more protracted time course, possibly of several months, during which the hepatitis may have been subclinical. Submassive necrosis and a clinical chronology consistent with ALF should be

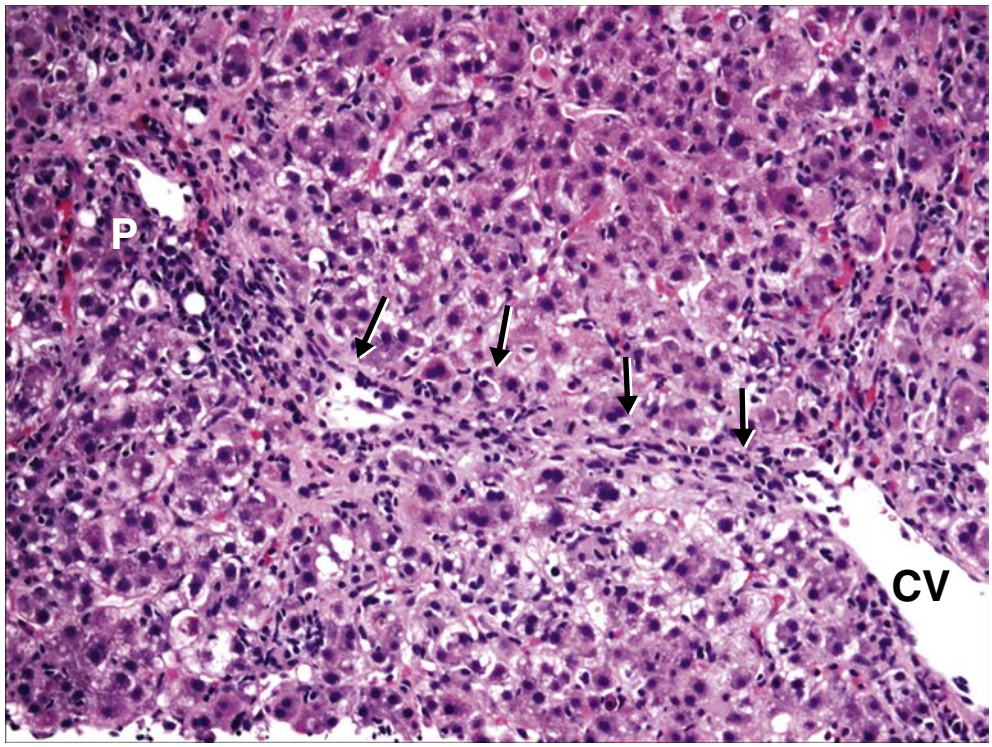


Fig. 4.7 Bridging hepatic necrosis. A narrow bridge of hepatocyte loss and inflammation (arrows) extends between the portal tract (P) and the central vein (CV) in this case of acute hepatitis. The liver parenchyma nearby shows extensive unrest and lobular disarray. Compare to the reticulin stain of a similar example in **Fig. 4.8**. (Needle biopsy, H&E.)

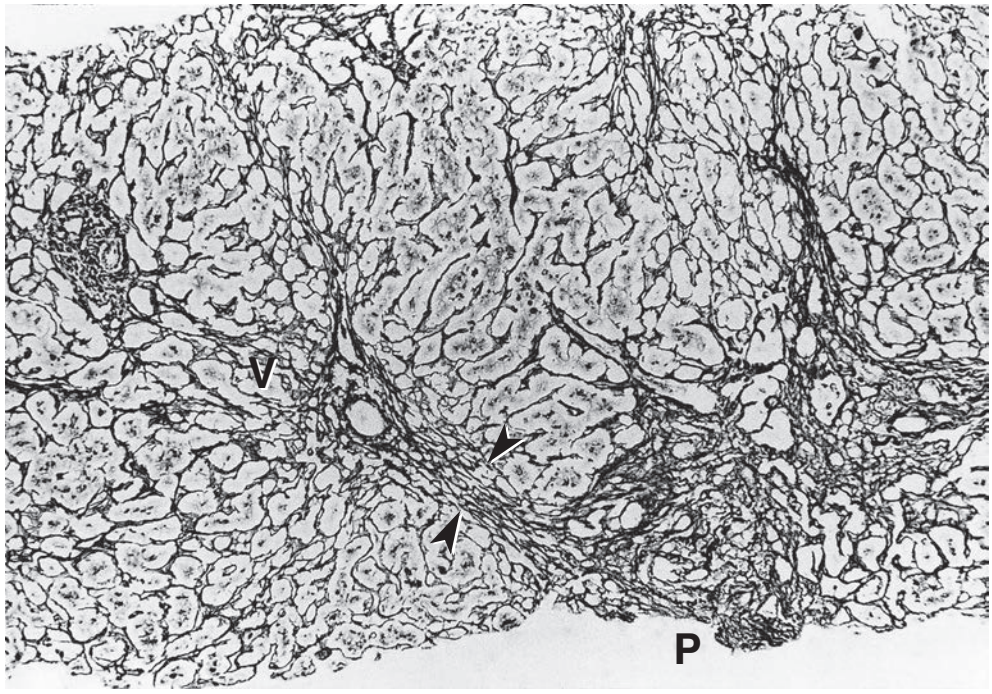


Fig. 4.8 Acute hepatitis with bridging necrosis. Collapsed reticulin here gives a false impression of chronic liver disease. A bridge or passive septum (arrowheads) links an expanded portal tract (P) with a terminal hepatic venule (V). (Needle biopsy, reticulin.)

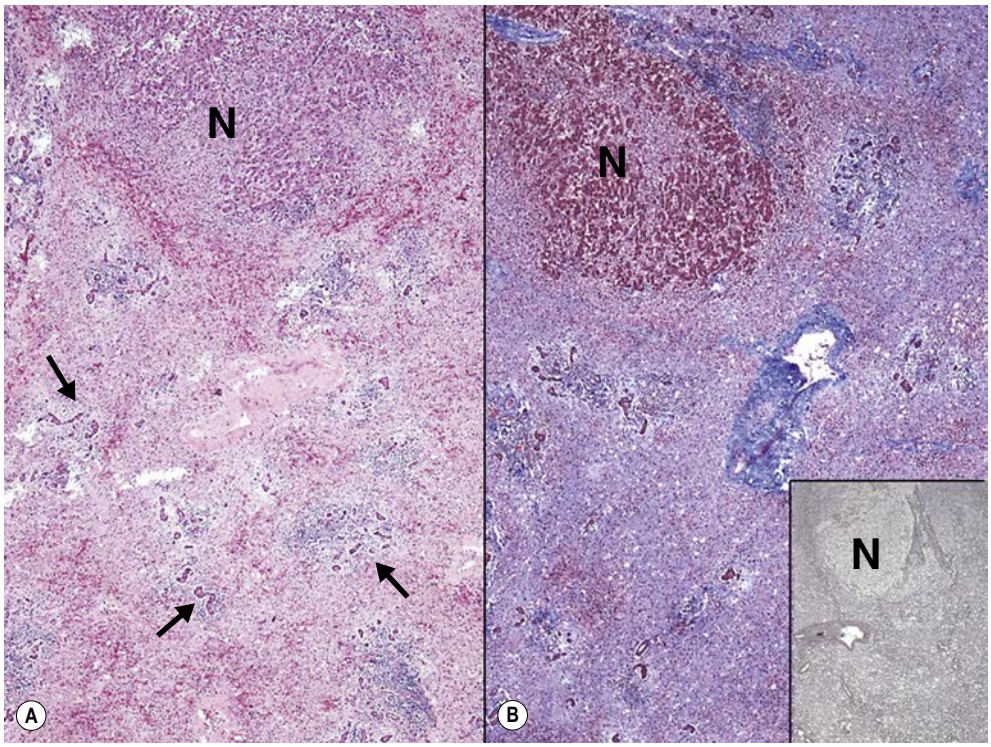


Fig. 4.9 Submassive necrosis. A: In this case of fulminant hepatitis, nearly all the liver parenchyma has disappeared due to massive necrosis. A ductular reaction is prominent (arrows). A few regenerative nodules were evident on gross examination of the explant liver and are also evident microscopically (N). **B:** Trichrome connective tissue stain highlights the extent of parenchymal necrosis, the ductular reaction, native portal tracts (bright blue) and very early fibrosis (light grey-blue) in the regions of collapse. An emerging regenerative nodule is present at top (N). Inset: Reticulin stain of the same field shown in **A** and **B** contrasts the reticulin collapse and condensation below and the regenerative nodule (N) at top. (Explant liver; **A**: H&E; **B**: trichrome stain; inset: reticulin stain.)

distinguished from *acute-on-chronic liver failure (ACLF)*, a condition seen in individuals who already have underlying cirrhosis or well-established chronic liver disease. ACLF is characterised by rapid progression of liver injury and one or multiorgan failure triggered by one or more factors (e.g. gastrointestinal tract haemorrhage, alcohol misuse) with a high risk of mortality within 3 months[13–15] (**see Ch. 10**). In such cases the pathologist's role, if liver biopsy has been obtained, is to verify the presence (or absence) of cirrhosis or chronic disease with advanced fibrosis. This determination has direct impact on subsequent clinical management decisions for the patient.

Interface hepatitis (piecemeal necrosis)

Interface hepatitis (piecemeal necrosis; **see Figs 9.3 and 9.4**) is a process of inflammation and erosion of the hepatic parenchyma at its junction with portal tracts or fibrous septa. The term *interface hepatitis* was introduced because the death of hepatocytes probably involves apoptosis rather than, or as well as, necrosis,[16–18] and because it takes place at the parenchymal–connective tissue interface. It is common in chronic viral hepatitis but is also found in other conditions. The inflammatory infiltrate is composed mainly of lymphocytes, with or without recognisable plasma cells, and is accompanied by fibrosis of the affected areas with new formation of collagens and other extracellular matrix components.[19] The process is sometimes referred to as classical or lymphocytic piecemeal necrosis

in order to distinguish it from biliary, ductular and fibrotic piecemeal necrosis, processes found in chronic biliary tract disease and described in the section on primary biliary cirrhosis in Chapter 5.

Cholestasis

In morphological terms, cholestasis is the presence of visible bile in tissue sections. It is also known as bilirubinostasis because the main component seen by light microscopy is bilirubin. Bile is rarely seen in normal liver, and then only in minute amounts; cholestasis should therefore be regarded as pathological. The location of the bile varies. The most common is in dilated bile canaliculi between hepatocytes. This canalicular form of cholestasis, sometimes called **acute cholestasis**, may be accompanied by bile accumulation in the cytoplasm of hepatocytes and Kupffer cells. Canalicular cholestasis is typically perivenular. By contrast, in patients with chronic biliary tract disease, bile may accumulate in periportal hepatocytes. This is also known as cholate stasis because abnormal bile salts are thought to contribute to its pathogenesis.

In large bile-duct obstruction in adults, bile is not usually visible under the microscope within canals of Hering, bile ductular structures or bile ducts, even though the biliary tree may be dilated. The most common cause of ductular cholestasis is sepsis. Dense bile is also visible in ductules and ducts in different forms of ductal plate malformation and in extrahepatic biliary atresia.

Canalicular cholestasis takes the form of bile plugs (bile thrombi) in dilated canaliculi (**see Fig. 5.2**). There is often brown or yellow pigment in nearby hepatocytes and Kupffer cells, but the distinction of this pigment from others such as lipofuscin and ceroid is not a serious practical problem; this is because the presence of bile in the canaliculi makes the diagnosis of cholestasis obvious. In general, cholestasis should only be diagnosed with great caution in the absence of bile plugs in canaliculi, although cytoplasmic liver-cell bilirubinostasis without canalicular bile is quite common after liver transplantation. The perivenular location of canalicular cholestasis is partly an artefact of paraffin embedding, but also reflects real functional differences between the various parts of the acinus.

The colour of bile under the microscope varies according to pigment concentration and the degree of oxidation. It may be dark brown, green or yellow, and is occasionally so pale as to make detection difficult at first glance. The van Gieson stain, which stains bilirubin green, may then be helpful (Fig. 4.10). Pale counterstaining, as commonly used in Perls' and Prussian blue methods for iron, also makes bile easier to see. Specific histochemical methods for bilirubin are rarely necessary in ordinary diagnostic work.

Box 4.1 Common causes of canalicular cholestasis

Obstruction to major bile ducts
Acute hepatitis
Cholestatic drug jaundice
Sepsis
Cholestatic syndromes

Box 4.2 Main causes of bland intrahepatic cholestasis

Drugs (e.g. contraceptive steroids)
Sepsis
Benign recurrent intrahepatic cholestasis
Cholestasis of pregnancy
Posttransplant bile flow impairment or rejection
Lymphomas

When acute cholestasis is prolonged, the relationship of hepatocytes to each other may undergo focal change. Instead of the normal arrangement of two or three hepatocytes around a small bile canaliculus, the number of cells is increased and the lumen of the canaliculus considerably enlarged. The new structures are called cholestatic rosettes (Fig. 4.11). The lumens of the rosettes are part of the biliary tree, but the bile may be lost during processing. Even apparently empty rosettes should therefore be regarded as an indication of cholestasis. Other hepatocellular changes in cholestasis are described in Chapter 5, in the section on large bile-duct obstruction. Very occasionally prolonged canalicular cholestasis is associated with the accumulation of copper and copper-associated protein, but this is much more characteristic of the chronic periportal form of cholestasis (discussed later). Canalicular cholestasis in perivenular areas is mainly seen in the conditions listed in Boxes 4.1 and 4.2. Cholestasis of less regular distribution is common in chronic liver diseases with severe hepatocellular dysfunction or with associated sepsis.

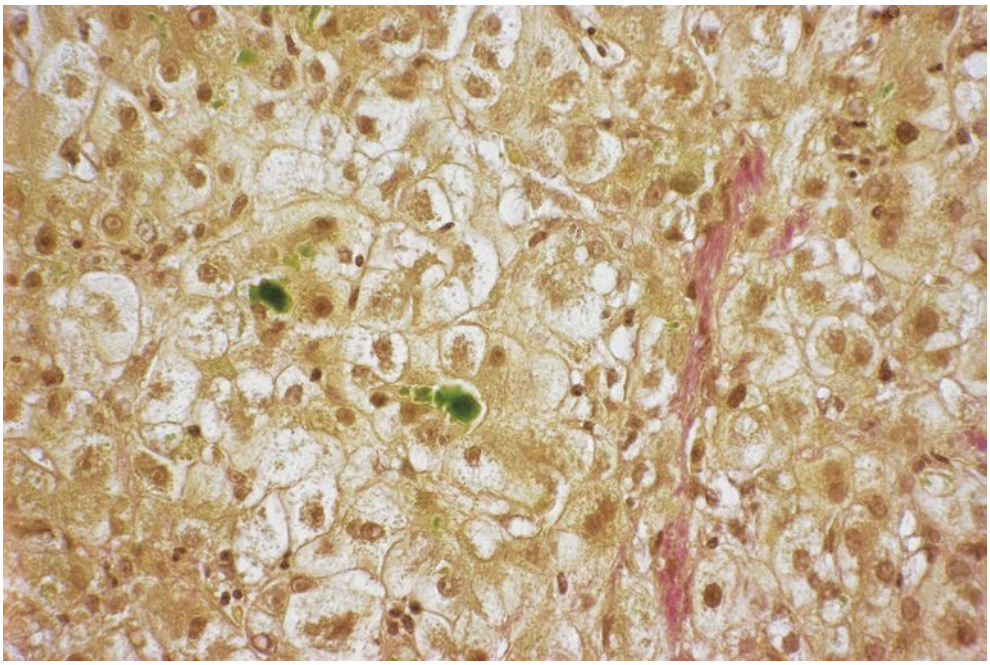


Fig. 4.10 Cholestasis. Bile thrombi in dilated canaliculi are stained bright green. The red material is collagen. (Needle biopsy, haematoxylin and van Gieson.)

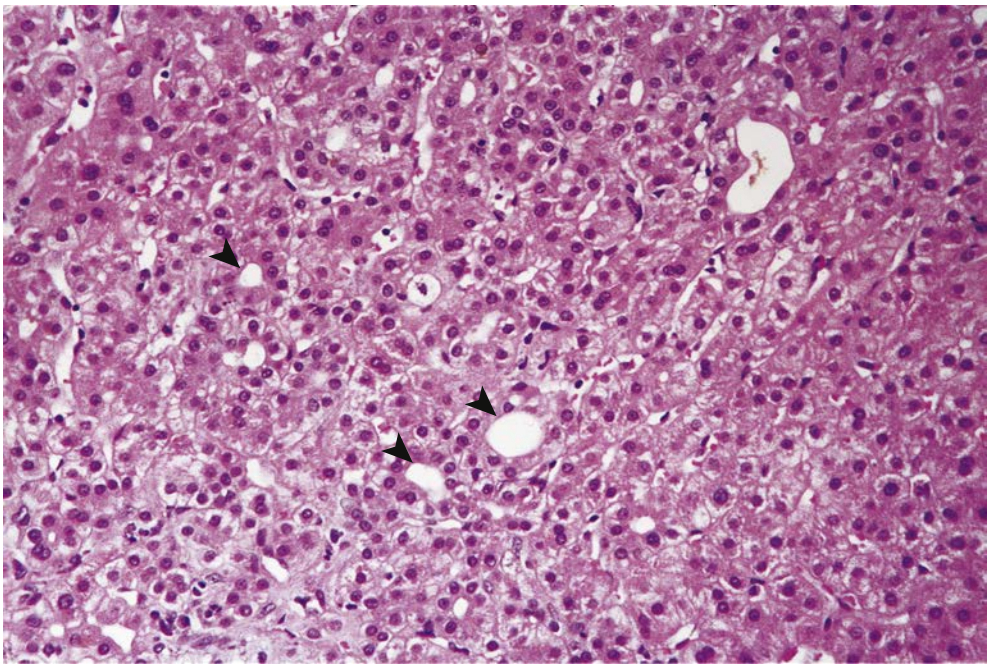


Fig. 4.11 Cholestasis. Several liver-cell rosettes, glandular formations around prominent lumens, are marked by arrowheads. (Wedge biopsy, H&E.)

Box 4.3 Decisions in the acutely jaundiced patient

- Are the patient's major bile ducts obstructed?
- Does the patient have an acute viral or drug-related hepatitis?
- Is there evidence for a diagnosis of sepsis?
- Does the patient have one of the intrahepatic conditions listed in **Box 4.2**?
- Does the patient have steatohepatitis?
- Does the patient have chronic liver disease with an acute exacerbation rather than acute liver disease?

Once cholestasis is identified, the pathologist's main concern should be determining its likely cause. Pertinent questions for consideration are listed in **Box 4.3**. The aetiology usually rests among four diagnostic categories: (1) large bile-duct obstruction; (2) disorders which affect the small, intrahepatic bile ducts; (3) hepatitis; and (4) conditions associated with bland cholestasis (e.g. sepsis, bile-salt transporter mutations). These can usually be distinguished by careful and methodical examination of abnormalities in the lobules and in the portal tracts (**Fig. 4.12**). Accurate histological diagnosis is important because correct treatment may depend upon it, and a wrong answer can lead to dangerous mismanagement. It has to be admitted, however, that the pathologist cannot always give a clear answer to the questions put by the clinician.

Ductular reaction

Because large bile-duct obstruction may require a surgical or endoscopic intervention, biopsies with cholestasis require careful inspection of the portal tracts for the triad of changes[20] that typically develops within several days of obstruction, collectively referred to as the **ductular reaction**: oedema of the portal tract connective tissue, proliferation of bile ductular structures at the edges of the oedematous portal tract stroma and scattered neutrophil infiltrates. The ductular structures which develop as a prominent feature in a variety of biliary and other conditions are believed to arise from periportal progenitor cells located in the canals of Hering[21,22] or possibly from bile-duct cells or transdifferentiated hepatocytes (**see Ch. 5**).[23,24] The ductular reaction can be viewed as a stereotypical periportal response to injury[25–27] which is exemplified by acute biliary obstruction, but which also occurs in several other pathological settings.

Certain features help in interpreting the diagnostic significance of the ductular reaction. In acute biliary obstruction, the ductular structures are arranged in parallel to the portal–parenchymal interface, associated with the portal oedema and scattered neutrophils previously mentioned (**Fig. 4.13A**). In chronic biliary tract diseases such as primary biliary cholangitis, the ductular profiles may lie at an angle to the interface or form convoluted tangles (**Fig. 4.13B**). Hepatocellular diseases may also act as a stimulus for the ductular reaction. In a minority of patients with acute hepatitis with much cholestasis, as seen, for example, in hepatitis A, a ductular reaction may accompany portal infiltrates of lymphocytes and plasma cells.[28a] The picture can mimic that of biliary obstruction, and the distinction requires careful consideration of the lobular changes. Ductular reaction is virtually always associated with neutrophils, so that the presence of these cells is not in itself evidence of bile-duct obstruction. Neutrophils are recruited as a result of production of various chemokines and other factors by the hepatic progenitor cell-derived ductular epithelilal cells.[28b] Ductular reaction also is seen in some examples of non-biliary cirrhosis in which the ductular structures are not necessarily limited to the margins of portal tracts or the septal–parenchymal interface, but extend to greater distances into the fibrous tissue (**Fig. 4.13C**). However, extensive ductular reaction accompanied by other features of chronic cholestasis suggests cirrhosis of biliary origin.

In panlobular necrosis (seen, for example, in patients with fulminant or subacute viral hepatitis, in severe drug hepatotoxicity or in autoimmune hepatitis), extensive loss of hepatocytes is often associated with an exuberant ductular reaction extending from periportal regions further inward and toward the centres of lobules (**Fig. 4.13D**). The ductular reaction is now considered a major participant in the process of bridging and more progressive fibrosis in chronic liver diseases, including chronic hepatitis B and C,[29–31] steatohepatitis[32] and haemochromatosis[33] (**see Fig. 9.7**). The ductular reaction may be unusually prominent

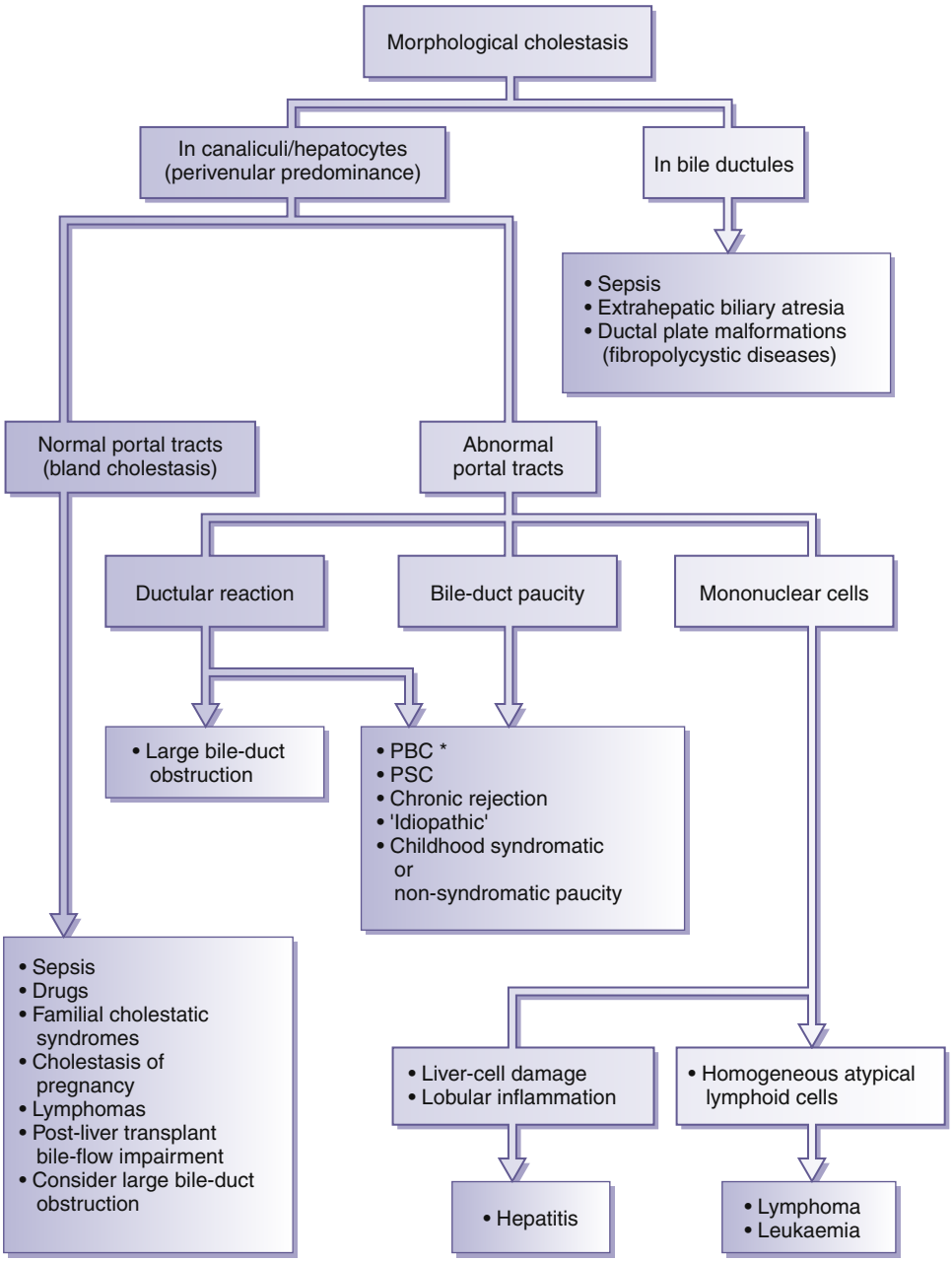


Fig. 4.12, Algorithmic approach to cholestasis. Once the site of cholestasis is identified pathologically, careful assessment of portal tracts and acinar changes allows the major differential diagnosis to be established. *In primary biliary cirrhosis (PBC), morphological cholestasis is usually only apparent in later, advanced disease. PSC, primary sclerosing cholangitis.

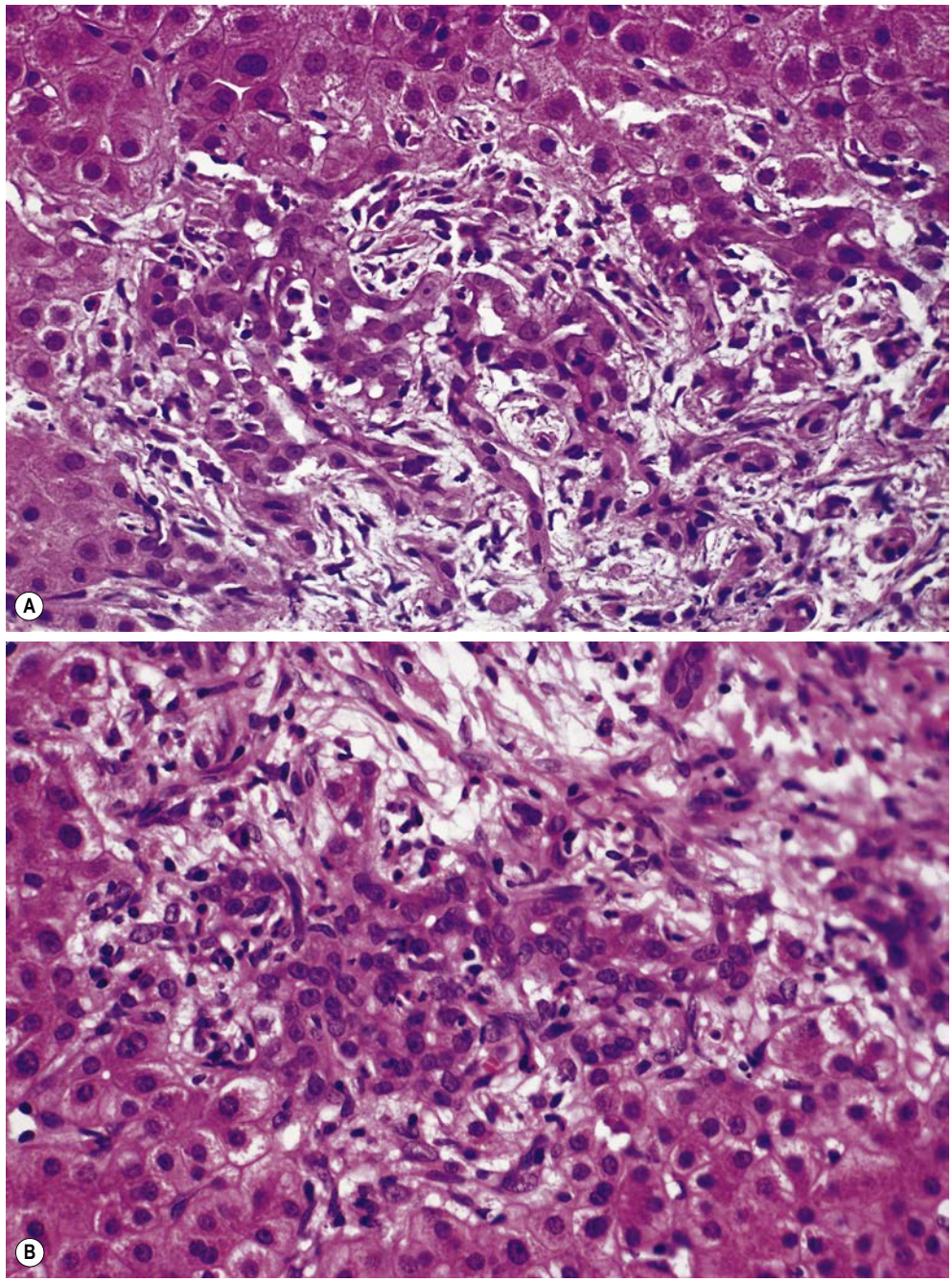


Fig. 4.13 The ductular reaction in different diseases. A: Ductular structures at the edge of the portal tract in bile-duct obstruction. **B:** Tangle of ductules in primary biliary cholangitis.

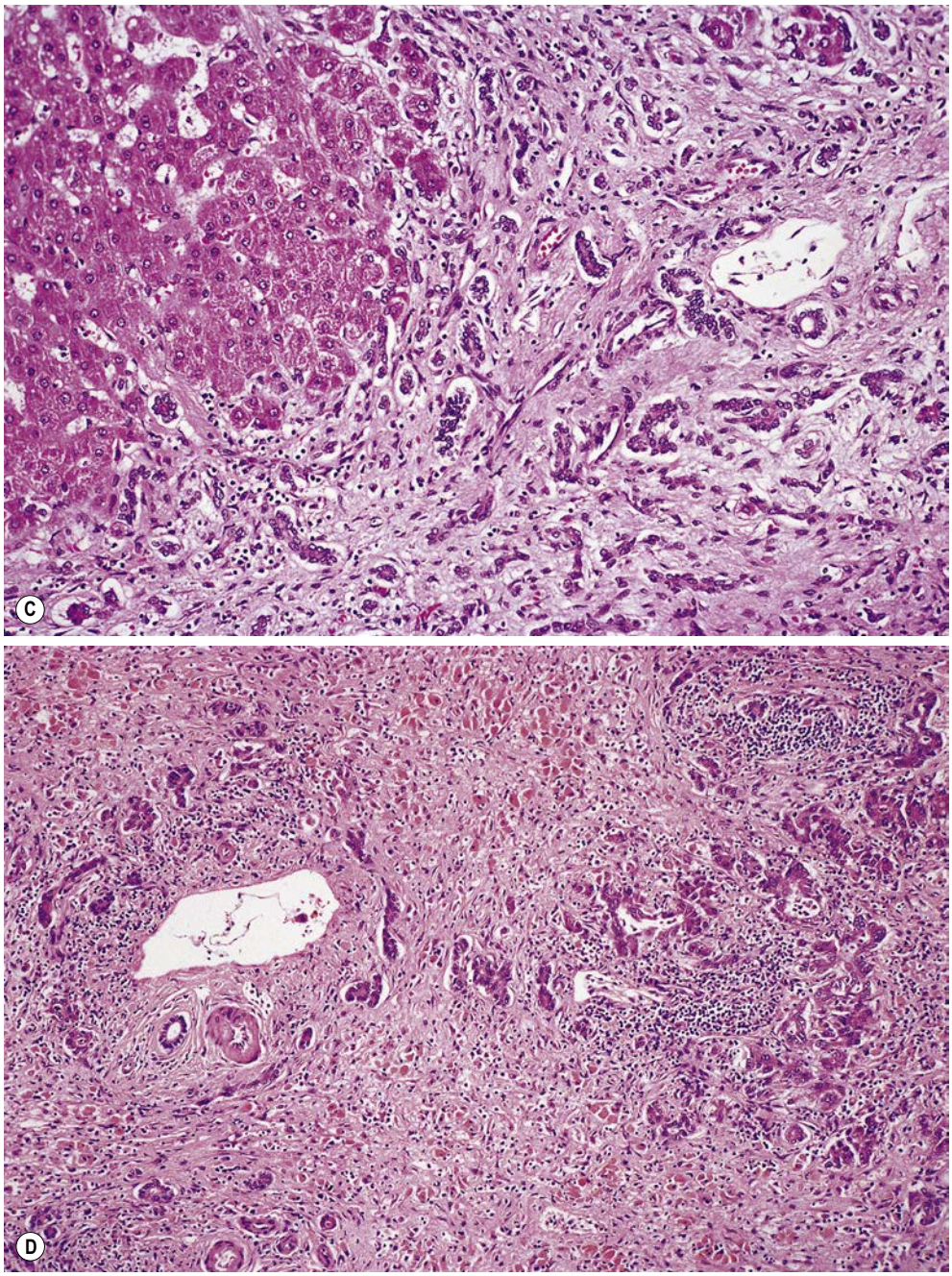


Fig. 4.13, cont'd
C: Non-biliary cirrhosis: ductular structures near the edge of the nodule and within the fibrotic portal tract. **D:** Multilobular necrosis: the duct-like structures probably reflect progenitor-cell activity in the absence of adequate hepatocellular regeneration. (H&E.)

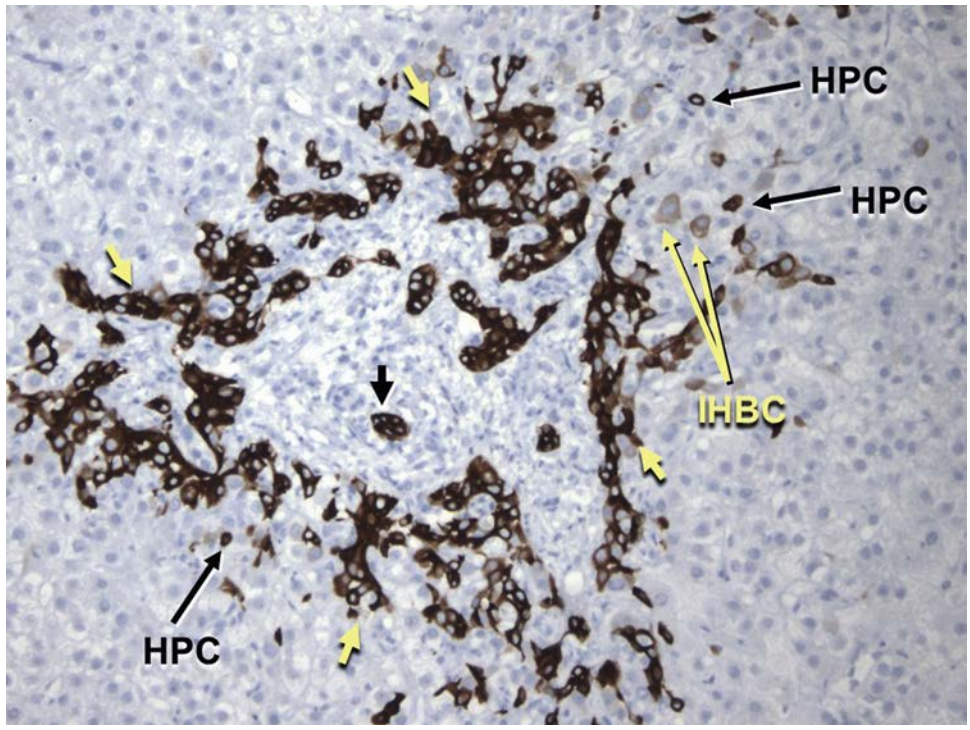


Fig. 4.14 The ductular reaction with cytokeratin 7 immunohistochemistry. In this case of large bile-duct obstruction numerous bile ductular structures have developed (short yellow arrows) and are more or less parallel to the portal–parenchymal interface. The native interlobular bile duct is near centre (short black arrow). A few periportal cells with less intense staining more closely resemble hepatocytes and represent intermediate hepatobiliary cells (IHBC), also termed 'biliary hepatocytes'. Small, round and darkly stained periportal cells are likely hepatic progenitor cells (HPC). (Needle biopsy, specific immunoperoxidase.)

in fibrosing cholestatic hepatitis, which develops in a minority of patients with recurrence of hepatitis B or C after liver transplantation (**see Figs 16.14 and 16.15**). In any situation in which the relative diagnostic importance of the ductular reaction must be established, immunostains for cytokeratin 7 or 19 are useful for highlighting the ductular structures (**Fig. 4.14 and see Figs 5.24 and 5.25**).

Chronic cholestasis (cholate stasis, pseudoxanthomatous change, precholestasis; **see Fig. 5.10**) is seen in chronic liver diseases, especially those involving the biliary tree, and is the result of interference with bile flow at the level of the portal tracts. Bile (i.e. bilirubinostasis) may or may not be obvious, and the lesion is more easily recognised by periportal hepatocellular swelling and pallor, and by the accumulation of copper and copper-associated protein in the affected cells. Mallory–Denk bodies may also be present. In some instances these are associated with an infiltrate of neutrophils, in which case the distinction from steatohepatitis must be made on the overall appearances, the periportal location and clinical context. The connective tissue adjacent to an area of chronic cholestasis is often oedematous. It may show a ductular reaction with mixed acute and chronic inflammatory cells which sometimes disrupts the limiting plates of hepatocytes around the portal tracts. The blurring of this margin has been likened to the features seen in classical interface hepatitis of chronic hepatitis (where a lymphoplasmacytic infiltrate blurs the portal tract-limiting plate margin), and has been referred to with terms such as 'biliary interface hepatitis' (formerly 'ductular piecemeal necrosis'). In chronic biliary tract disease, the ductular structures and associated neutrophils are helpful for recognising the presence and role of

the ductular reaction. Chronic cholestasis, unlike acute canalicular cholestasis, is not necessarily associated with clinical jaundice or a high level of serum bilirubin, but the serum alkaline phosphatase level is characteristically raised.

Loss of interlobular bile ducts is a key feature of several diseases in childhood and adult life. These are sometimes referred to as *vanishing bile-duct syndromes*. The principal causes in children are syndromatic and non-syndromatic paucity of intrahepatic bile ducts, α_1-antitrypsin deficiency and early-onset sclerosing cholangitis. Some uncommon familial cholestatic syndromes and Langerhans-cell histiocytosis should also be considered (**see Ch. 11**). In adults (**see Table 5.1**) the most common causes are primary biliary cholangitis, primary sclerosing cholangitis, graft-versus-host disease and chronic liver graft rejection.

In assessing duct loss it is important to bear in mind that not every small portal tract is seen to contain a bile duct in the plane of section. In a study of normal human liver biopsies,[34] 7% of sectioned portal tracts did not contain a bile duct. For confident assessment of duct numbers a biopsy must therefore contain several portal tracts. Loss of ducts is accompanied in many, but not all, cases by the features of chronic cholestasis outlined earlier. This depends on the extent of duct loss, the underlying aetiology and the degree of fibrosis. A significant ductular reaction develops in some conditions of bile-duct loss (primary biliary cholangitis, primary sclerosing cholangitis) but not others (Alagille's syndrome in children,[35] chronic liver graft rejection[36]).

Granules of the **copper-associated protein** metallothionein can be stained by several methods, including orcein and Victoria blue. They are usually positive with periodic acid–Schiff (PAS) staining after diastase digestion. Their most common location is in periportal hepatocytes or, in cirrhotic livers, in hepatocytes at the periphery of nodules. This reflects the inability of the hepatocytes to excrete copper efficiently. Some granules can be seen in cirrhosis of any cause, but large amounts should lead to a suspicion of chronic biliary tract disease or intrahepatic cholestasis.[37] Copper itself is usually demonstrable in the same location, and there may be other features of chronic cholestasis, such as ductular proliferation, neutrophils, intercellular fibrosis and oedema. A few granules of copper-associated protein are sometimes seen deeper within the acini in prolonged acute cholestasis.

Copper-associated protein also accumulates in Wilson's disease, as discussed in **Chapter 14**. As a rule, neither the protein nor copper itself is demonstrable by staining in the early stages of the disease. When cirrhosis develops in Wilson's disease, some nodules may be rich in copper-associated protein and copper (although one may be demonstrable without the other), while others are negative. The copper and the protein are usually diffusely distributed throughout a nodule, in contrast to their location in chronic cholestasis.

Differential diagnosis of individual findings

The selected features illustrated and described in the following are discussed in other chapters of this book but occur with sufficient frequency or in distinctive contexts as to merit highlighting here.

Bile-duct damage

The presence of damage to intrahepatic bile ducts is usually signalled by the presence of duct epithelial changes accompanied by adjacent portal tract inflammatory cell infiltrates. Damage to intrahepatic bile ducts has many possible causes; prominent examples include primary biliary cirrhosis, idiosyncratic drug toxicity and acute cellular rejection following liver transplantation. Injured bile ducts demonstrate a variety of epithelial abnormalities, including intraepithelial inflammatory cells, epithelial stratification, vacuolisation, necrosis and attenuation, and altered nuclear polarity (**Fig. 4.15**). The inflammation is chiefly lymphocytic, with variable numbers of plasma cells and occasional neutrophils. Eosinophils may be prominent, particularly in primary biliary cholangitis and with certain hepatotoxic drugs. The most extreme damage may result in complete destruction of the duct epithelium resulting in widespread bile duct loss (*ductopenia*).

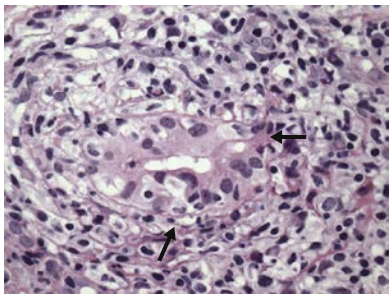
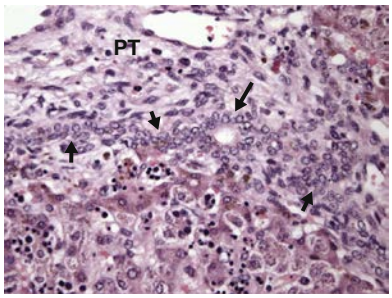


Fig. 4.15 Bile-duct damage.
The epithelium of the bile duct at centre is infiltrated by lymphocytes and shows altered nuclear polarity, focal vacuolisation and nuclear stratification. The affected bile duct is an example of a 'florid bile duct lesion' seen in primary biliary cirrhosis. The pink basement membrane surrounding the duct has ruptured (arrow below) and has been breached by lymphocytes (arrow at 3 o'clock). (Needle biopsy, H&E.)

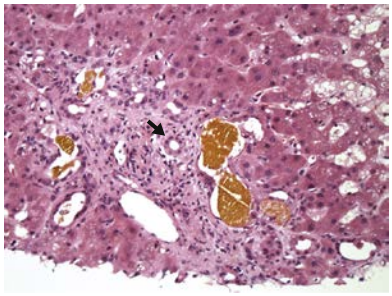
Bile-duct plate



The bile-duct plate is a normal feature of intrahepatic bile-duct tubulogenesis in the growing fetal liver.[38] It may take the form of a single- or double-layered flattened cuboidal epithelial layer surrounding all or part of the loosely organised stroma of the developing portal tracts (**Fig. 4.16**). Its significance lies in its recognition in fetal liver specimens and in understanding its relationship to liver diseases characterised by ductal plate malformations such as congenital hepatic fibrosis (**see** Ch. 13) which represent abnormalities of bile-duct plate remodelling.

Fig. 4.16 The bile-duct plate.
In this 19-week fetus the flattened cuboidal epithelium of the bile-duct plate (small arrows) surrounds the circumference of the portal tract (PT) seen at top. Early bile-duct tubulogenesis has been initiated (large arrow). (Postmortem liver, H&E.)

Bile ductular cholestasis



In adults, inspissated concretions of bile localised within periportal bile ductular structures is chiefly seen in sepsis (**Fig. 4.17**). Many, if not most, portal tracts are affected, but the native bile ducts usually do not contain bile. In the neonatal liver biopsy this lesion may be seen in extrahepatic biliary atresia and in α_1-antitrypsin deficiency (although in these disorders the ductular bile may be very focal and is usually in smaller bile plugs or inspissates).

Fig. 4.17 Bile ductular cholestasis. Pools of inspissated bile are present in dilated periportal bile ductular structures. This distribution of cholestasis is characteristically seen in sepsis. Note that the native bile duct (arrow) does not contain bile. (Needle biopsy, H&E.)

Ceroid-laden Kupffer cells

Recent hepatic necroinflammatory activity (e.g. acute or chronic hepatitis or ischaemic injury) is often associated with intrasinusoidal collections of tan-brown-staining, ceroid-laden Kupffer cells (**Fig. 4.18**). The pigment is rich in oxidised lipids, is found within Kupffer cell lysosomes and represents phagocytic debris derived from the cell membranes and other organelles of necrotic hepatocytes. Ceroid-laden Kupffer cells are typically more prominent in centrilobular regions (acinar zones 3), stain positively with diastase-PAS and retain their tan-brown colour on iron stain. The pigment should not be misconstrued as haemosiderin (which on H&E stain appears more glassy and refractile), although small amounts of haemosiderin are sometimes present in acute hepatitis. Recent episodes of obstructive jaundice with cholestasis occasionally lead to similar-appearing pigment in Kupffer cells.

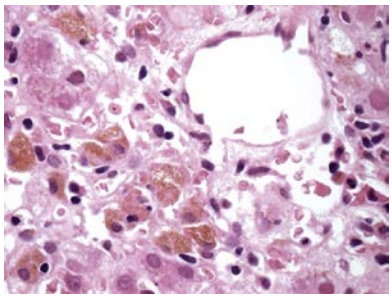


Fig. 4.18 Ceroid-laden Kupffer cells.
Recent necroinflammatory activity near the efferent vein at top has resulted in liver-cell dropout and intrasinusoidal collections of enlarged Kupffer cells with tan, granular pigment (phagocytic debris in lysosomes). (Needle biopsy, H&E.)

Sinusoidal congestion

Sinusoidal congestion is often centrilobular (acinar zone 3) due to obstruction of efferent venous outflow returning to the heart in patients with heart failure (**Fig. 4.19**; the so-called nutmeg liver on gross examination), or other causes of *hepatic venous outflow obstruction* (**see Ch. 12**). Sinusoidal dilatation with liver-cell plate atrophy may accompany the congestion, and if obstruction is chronic, there may also be perivenular and perisinusoidal fibrosis (*cardiac sclerosis*). In the liver allograft biopsy following liver transplantation, centrilobular congestion with associated lymphocytic central vein and sinusoidal endotheliitis, hepatocyte drop-out and variable mild sinusoidal dilatation are diagnostic of *central perivenulitis* as a manifestation of acute cellular rejection. Diffuse sinusoidal congestion is seen in the *congestive hepatopathy* of sickle-cell disease, whereas periportal congestion, haemorrhage, fibrin thrombi and hepatocyte necrosis are features seen in eclampsia (**see Ch. 15**).

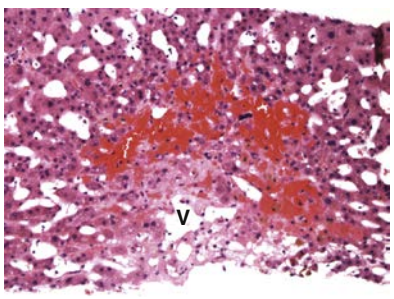


Fig. 4.19 Sinusoidal congestion.
Sinusoids near the efferent vein (V) are congested and dilated in this biopsy from a patient with long-standing history of heart failure. Mild fibrosis is seen above the vein lumen, consistent with chronic venous outflow obstruction. (Needle biopsy, H&E.)

Erythrophagocytosis

Kupffer cell erythrophagocytosis (**Fig. 4.20**) is an unusual histological finding seen most often in systemic viral infections and in association with haemophagocytic lymphohistiocytosis[39] (HLH) and its variant form macrophage activation syndrome (MAS). HLH and MAS usually develop in the setting of an underlying acquired condition such as rheumatic disease, systemic viral infection (herpes simplex and Epstein–Barr virus commonly) or malignant lymphoma, or there may be a known genetic cause,[40] with defective inflammatory cell granule function and/or release. Activated lymphocytes in HLH and MAS may infiltrate the portal tracts and sinusoids, sometimes causing bile-duct damage or histological changes resembling chronic hepatitis, including formation of apoptotic bodies (**see Ch. 15**).

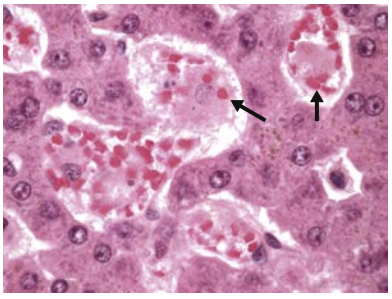
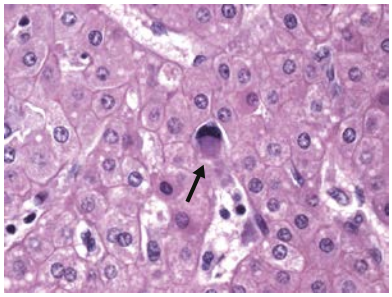


Fig. 4.20 Erythrophagocytosis.
Erythrocytes are readily seen within sinusoidal Kupffer cells (arrows) in this case of suspected haemophagocytic lymphohistiocytosis. (Needle biopsy, H&E.)

Extramedullary haemopoiesis



Extramedullary haemopoiesis (EMH) is often present in neonatal liver biopsies and may be seen in adults when bone marrow is replaced by neoplasm or in myelofibrosis. Certain primary liver tumours also feature EMH, particularly hepatoblastoma and hepatocellular adenoma. Congested liver and allograft liver biopsies following liver transplantation are other settings for EMH. **Isolated megakaryocytes** may be seen in any of the preceding conditions (**Fig. 4.21**). They are also occasionally identified in cirrhosis or nodular regenerative hyperplasia. Dysmature sinusoidal megakaryocytes are seen in transient abnormal myelopoiesis associated with Down syndrome.[41]

Fig. 4.21 Isolated sinusoidal megakaryocyte.
A solitary megakaryocyte (arrow) is present in a sinusoidal space in this biopsy specimen of hepatocellular adenoma. The constituent hepatocytes of the tumour appear benign and grow in thickened plates. (Needle biopsy, H&E.)

Perisinusoidal fibrosis

Fibrosis within the space of Disse (perisinusoidal fibrosis) in centrilobular regions (acinar zones 3) may be seen in steatohepatitis (**see Ch. 7**) or, in the absence of steatosis, hepatocyte ballooning or evidence of steatohepatitis, as late residua following prior episodes of steatohepatitis. The histological differential diagnosis includes long-standing cardiac failure (*cardiac sclerosis*) and other conditions associated with chronic hepatic venous outflow obstruction (**Fig. 4.22**); previous episodes of centrilobular necroinflammation in the variant histological form of autoimmune hepatitis (**see Fig. 9.20**) and in liver allografts after liver transplantation, as sequelae of previous episodes of rejection with central perivenulitis (**see Ch. 16**). Non-zonal perisinusoidal fibrosis may be seen in certain diabetics with *diabetic hepatosclerosis* (**see Ch. 7**). In liver transplant recipients with recurrent hepatitis C virus (HCV) infection, perisinusoidal fibrosis in periportal[42] and lobular[43a] regions may be associated with more severe disease, including fibrosing cholestatic hepatitis[43a] (**see Ch. 16**). Whether centrilobular or elsewhere, perisinusoidal fibrosis should raise the question of possible hypervitaminosis A and inquiry into current and recent medications or the use of herbal/supplemental agents.[43b]

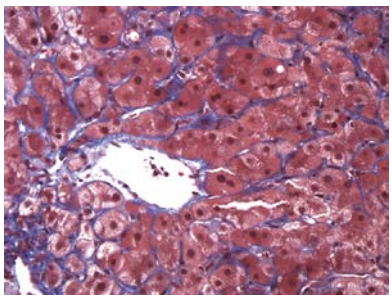


Fig. 4.22 Perisinusoidal fibrosis.
The central vein shows a surrounding network of perisinusoidal and pericellular fibrosis typical of the 'chicken-wire' fibrosis associated with alcoholic and non-alcoholic steatohepatitis. Residual fibrosis of this type may be present after resolution of steatosis and steatohepatitis. (Needle biopsy, H&E.)

Inflammatory cell infiltration

Neutrophils

Neutrophils are most numerous in portal tracts in large bile-duct obstruction, and in any condition in which there is an extensive ductular reaction (discussed earlier). In ascending cholangitis they are found in the lumens and walls of bile ducts. In intrahepatic or extrahepatic sepsis there may be neutrophils around ducts and bile within their lumens. Neutrophils are also seen in the sinusoids. A few neutrophils in a predominantly lymphocytic–plasmacytic portal infiltrate are common in acute hepatitis from any cause, but predominance of neutrophils suggests possible drug-related liver injury and in the appropriate clinical setting may be an indication for exclusion of hepatitis E virus infection (**see Ch. 6**).

Diffuse infiltration of the parenchyma by neutrophils is unusual. It may represent a classical acute inflammatory response to extensive tissue destruction from any cause, such as an adjacent abscess or possible necrotic neoplasm. Localised infiltrates are found in steatohepatitis, especially when alcohol related. However, a mainly lymphocytic infiltrate does not exclude the

diagnosis of steatohepatitis if other features such as hepatocyte ballooning or Mallory-Denk bodies are present (**see Ch. 7**). Focal accumulations of neutrophils (microabscesses) are a feature of cytomegalovirus infection (**see Fig. 16.13**) and of perfusion injury in liver grafts. They are also seen in many other complications of liver transplantation, though usually in smaller numbers than in cytomegalovirus infection.[44] Clusters of neutrophils may be found within sinusoids in any wedge biopsies taken in the course of surgery (**see Fig. 3.12**), and should not then be taken as indicating specific hepatic pathology.

Eosinophils

Portal infiltrates in many different liver diseases include occasional eosinophils, and their presence does not necessarily imply drug hypersensitivity or toxicity. Portal tracts often show a few eosinophils accompanying lymphocytes and plasma cells in chronic viral and autoimmune hepatitis. They are common in primary biliary cirrhosis and are occasionally abundant.[45] After liver transplantation they are one of the manifestations of cellular rejection.[46] An infiltrate with very prominent eosinophils suggests drug toxicity, systemic conditions with eosinophilia, parasitic disease or eosinophilic gastroenteritis.[47] Focal accumulations of eosinophils are seen in the parenchyma within some granulomas, notably those due to parasites. Neonatal liver biopsies may show abundant eosinophils within portal tracts and periportal sinusoids as constituents of normal EMH.

Plasma cells

Portal and acinar plasma cell infiltrates are often striking in autoimmune hepatitis, but they may also be seen in acute or chronic viral hepatitis. They are sometimes abundant in hepatitis A. Plasma cells form an important component of the portal infiltrates of primary biliary cirrhosis. Numerous plasma cells in portal tracts with features of biliary tract obstruction, including periductal 'onion-skin' fibrosis, should raise the possibility of IgG4-related sclerosing cholangitis (**see Ch. 5**). Large numbers of plasma cells in liver allograft biopsies may be seen in recurrent autoimmune hepatitis, *de novo* autoimmune hepatitis and as a manifestation of late allograft rejection. Prior to the availability of direct-acting antiviral therapy, HCV-positive liver transplant recipients who were treated with interferon sometimes developed a severe, plasma cell-enriched 'alloimmune' type of rejection (**see Ch. 16**).

Lymphoid aggregates and follicles

Lymphoid structures (aggregates and follicles) may develop within portal tracts in several chronic liver diseases; the chief differential diagnosis includes chronic hepatitis, primary biliary cholangitis and primary sclerosing cholangitis. Lymphoid aggregates are found considerably more often than follicles with germinal centres. Lymphoid aggregates are usually evident on low power as discrete, dense collections of lymphocytes that are distinct from the more dispersed inflammation in the remainder of the portal tract (**Fig. 4.23**). In chronic hepatitis, aggregates are most often located adjacent to interlobular bile ducts, or they may surround ducts, occasionally with resultant duct injury, but without bile duct loss. They are very common in chronic hepatitis C,[48] but less often present in chronic hepatitis B and autoimmune hepatitis. Following anti-viral therapy with sustained viral response, these lymphoid structures may still be evident in biopsy or explant specimens, despite seronegativity for HCV RNA. In primary biliary cholangitis they represent the 'tombstones' at sites of prior bile duct destruction. In primary sclerosing cholangitis they are a component of the ongoing acute and chronic periductal

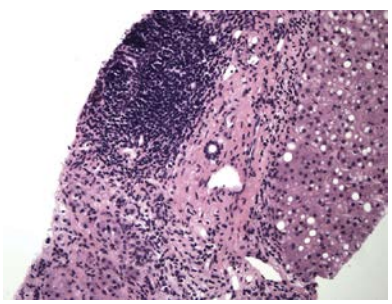


Fig. 4.23 Portal lymphoid aggregate.
This case of chronic hepatitis C shows a dense lymphoid aggregate in the portal tract, to the left of the interlobular bile duct. A much milder and dispersed lymphocytic infiltrate is present in the remainder of the portal connective tissue. (Needle biopsy, H&E.)

inflammation involving the large and small bile ducts. Diffuse, multifocal portal tract lymphoid structures may require further investigations to exclude lymphoma.

Abnormal macrophage pigment

Tan Kupffer cell pigment within sinusoidal Kupffer cells may represent phagocytic debris following recent necroinflammatory activity, as discussed earlier, or may represent biliary material after an episode of cholestasis or haemosiderin derived from erythrocyte breakdown. The latter pigment is positive on iron stain, while the former pigments usually are diastase PAS positive. Granular black haemozoin pigment (derived from haemoglobin breakdown) within sinusoidal Kupffer cells and/or portal macrophages is seen in malaria and in schistosomiasis (**see Ch. 15**). A similar black pigment occasionally is seen in individuals who have received gold salts or total knee or hip titanium–aluminium prosthetic replacements.[49]

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CHAPTER

5 Biliary Disease

Introduction

There are many sites along the biliary tree where bile flow may be interrupted, from the bile canaliculi and smallest intrahepatic ducts to the large bile ducts and duodenum (**Fig. 5.1**). Damage or obstruction at these various sites may result in visible bile in histological sections (cholestasis), altered bile-duct morphology, changes within the portal tracts and periportal parenchyma, or combinations of these. Diseases of the larger ducts must be distinguished from diffuse intrahepatic diseases because of different clinical management, and liver biopsy is often helpful in this respect. However, diseases of large bile ducts, outside and within the liver, share pathological features and may be amenable to similar forms of treatment; for this reason the term *extrahepatic biliary obstruction* is not used in this chapter. Carcinoma of the main hepatic ducts, for example, may be situated wholly within the liver, yet lead to the changes of large-duct obstruction. This chapter discusses these changes, along with the pathology of primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), the diagnostic problem of overlap with autoimmune hepatitis (AIH) and several bile-duct paucity disorders.

Cholestasis

The term *cholestasis* in clinical and pathological usage refers to impairment of bile flow. Under the light microscope, cholestasis (sometimes called *bilirubinostasis*) is defined as the presence of bile pigment within bile canaliculi, hepatocytes and other sites. It is the morphological correlate of clinical jaundice. Cholestasis is an important finding in large bile-duct obstruction or in extensive intrahepatic bile-duct disease, but may also accompany the parenchymal damage in certain types of hepatitis. Pure (bland) cholestasis as an isolated lesion requires consideration of several possible aetiologies (**Box 5.1**), which may not be distinguishable by light microscopy alone. For example, in neonatal and childhood jaundice, cholestasis may result from mutations in bile-salt transport proteins on the canalicular membrane[1] or from mitochondriopathies,[2] problems discussed further in **Chapter 13**. In adults, drug hepatotoxicity, circulating endotoxin in septicaemia[3] (**Fig. 5.2**) and cytokine release from extrahepatic lymphoma[4] are further examples of functional disorders of bile secretory physiology that may lead to intrahepatic cholestasis (discussed further in **Ch. 4**). The pathologist's first priority when cholestasis is present, nevertheless, is careful examination of the portal tracts for possible changes of mechanical large bile-duct obstruction, which are described in the following section.

Box 5.1 Causes of intrahepatic cholestasis

Septicaemia
Drug hepatotoxicity
Bile-salt transporter mutations (e.g. Byler disease)
Extrahepatic lymphoma
Mitochondriopathies (e.g. Navajo neurohepatopathy)
Early large bile-duct obstruction

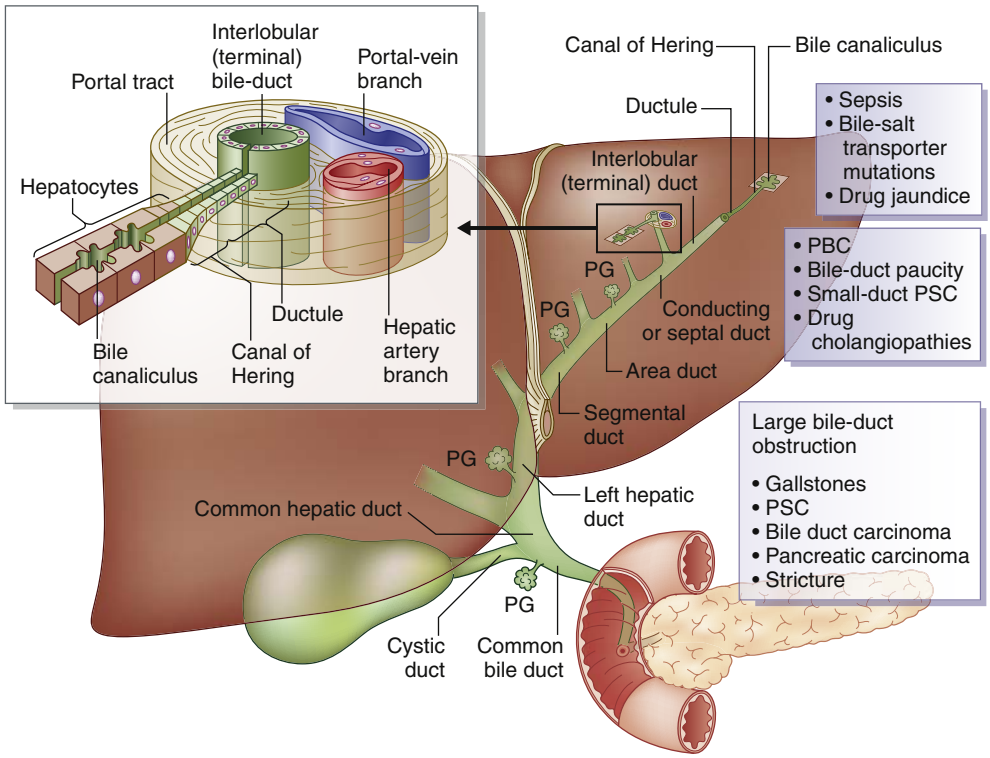

Fig. 5.1 The biliary tree. The ramifying structures of the biliary system are shown schematically. The large segmental and area ducts have peribiliary glands (PGs). The finer branches are shown in an enlargement at upper left. Boxes at right show examples of biliary disease at the specific levels affected. PBC, Primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

Large bile-duct obstruction

Biopsies from patients with large-duct obstruction are much less often seen than formerly because of improved imaging methods. However, the pathologist needs to be able to recognise the characteristic changes, especially following liver transplantation. From the first weeks of obstruction there is cholestasis in perivenular areas; that is to say, bile is visible under the microscope in the form of bile thrombi (bile plugs) in canaliculi and as yellow-brown pigment in hepatocytes and Kupffer cells (Fig. 5.3). The presence of canalicular bile thrombi distinguishes cholestasis from other pigmentations (**see** Table 3.1). Kupffer cells in cholestatic areas are enlarged and pigmented, containing both bile and diastase-resistant periodic acid–Schiff (PAS)-positive material. In recovering obstruction the Kupffer-cell changes persist while bile thrombi become smaller and less numerous. Finally, as in residual acute hepatitis, a few diastase–PAS-positive Kupffer cells may provide the only histological evidence of a recent episode of jaundice.

At first the hepatocytes in areas of cholestasis show little change, but with time they often become swollen. Their nuclei increase in size and number, and a few apoptotic bodies and mitoses may be seen, indicating increased cell turnover. Individual hepatocytes or small groups of cells undergo **feathery degeneration**, characterised by rarefied and reticular cytoplasm (Fig. 5.4). The lesion is focal, and the affected cells are typically surrounded by more or less normal hepatocytes. Feathery degeneration may be difficult to distinguish from the ballooning degeneration of hepatitis (**see** Fig. 6.2) or following liver transplantation, but in ballooning the cytoplasm is often granular rather than feathery and the lesion is more widespread in the lobule.

In a minority of patients with obstructed ducts, **bile extravasates, bile infarcts** and/or **bile lakes** develop (Fig. 5.5). These lesions are considered relatively pathognomonic of

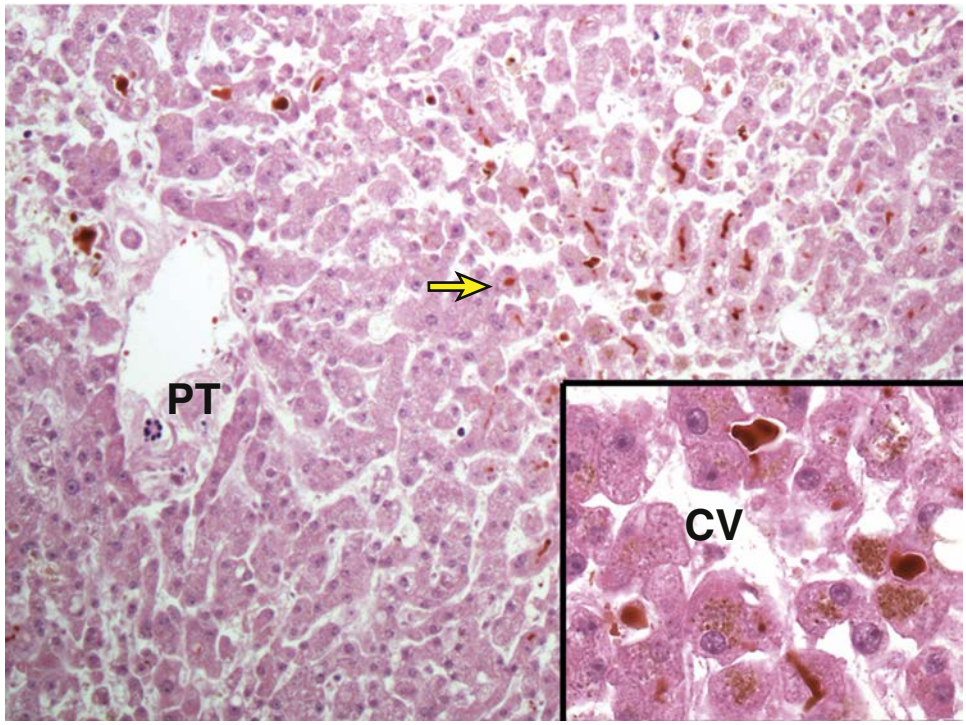

Fig. 5.2 Cholestasis in septicaemia. The centrilobular region (yellow arrow) shows prominent cholestasis in this patient who died of Gram-negative septicaemia. Note the normal portal tract (PT) at left which shows no features of biliary obstruction such as oedema, ductular reaction or neutrophil infiltrates. Inset: Liver parenchyma around the central vein (CV) shows prominent bile within hepatocytes and in cross- and longitudinal sections of bile canaliculi. (Postmortem liver, H&E.)

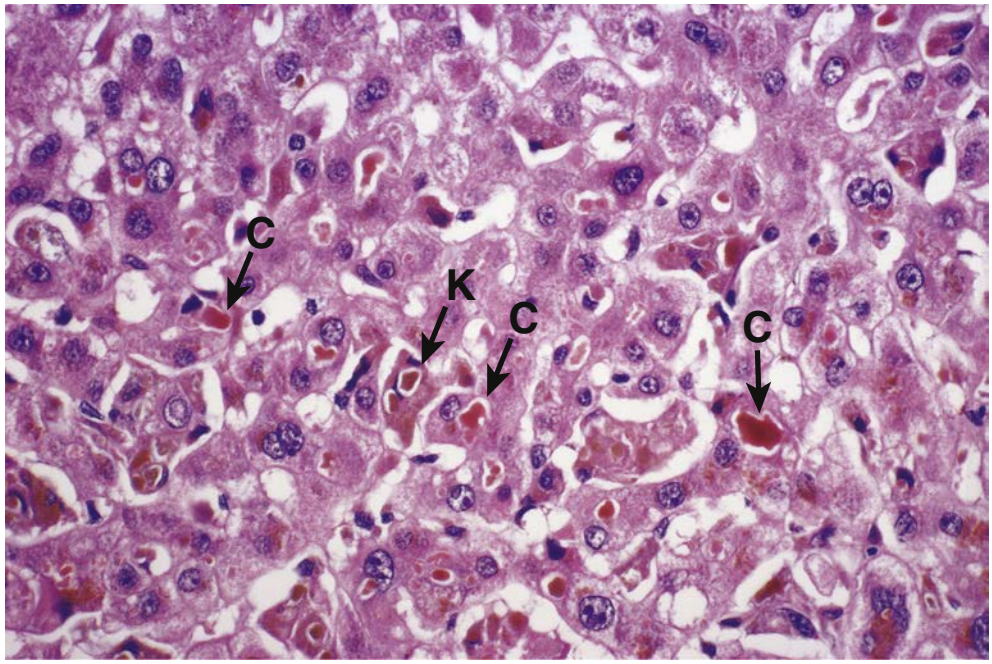

Fig. 5.3 Cholestasis. Bile is seen in the form of bile thrombi (bile plugs) in dilated canaliculi (C), as well as in Kupffer cells (K). (Needle biopsy, H&E.)

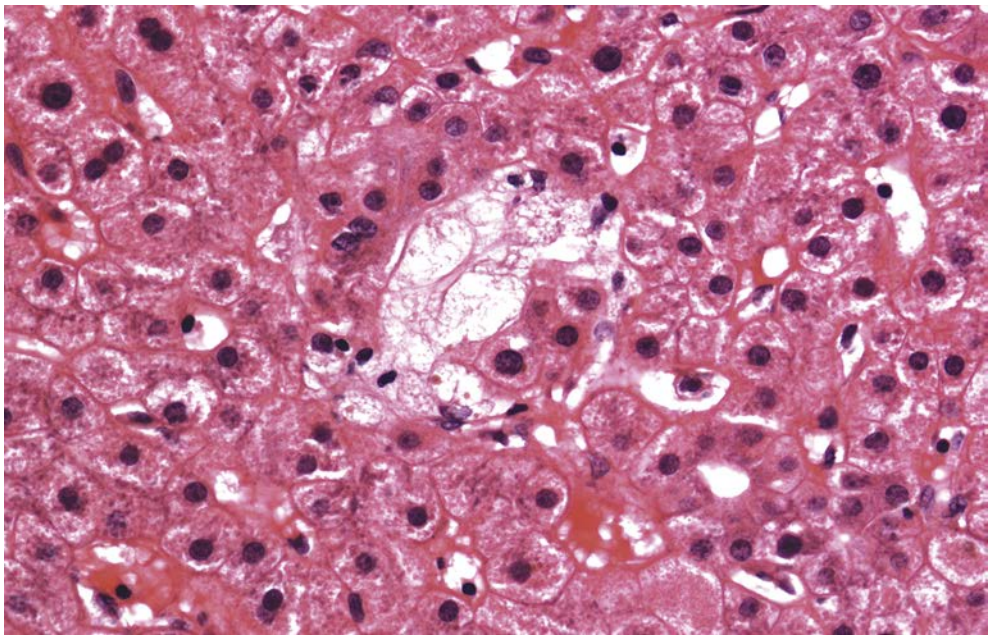

Fig. 5.4 Cholestasis. Small groups of swollen hepatocytes at centre have undergone feathery degeneration. Adjacent hepatocytes appear normal. (Wedge biopsy, H&E.)

large bile duct obstruction. **Bile infarcts** form, apparently, because of obstruction-related progressive increases in canalicular bile acid levels which rupture the apical canalicular membrane causing a canalicular bile-sinusoidal shunt and subsequent hepatocyte damage.[5,6] These are substantial areas of hepatocellular degeneration or death containing pale or bile-stained hepatocytes or discrete rounded cells that are difficult to distinguish from macrophages. There are variable amounts of bile and fibrin, the latter often abundant. Reticulin fibres become progressively more difficult to demonstrate. Bile eventually leaches out of the infarct to leave a barely pigmented and scarcely stained lesion containing the ghosts of hepatocytes. Small bile infarcts may be found in severe cholestasis from any cause; larger infarcts such as the one shown in **Fig. 5.5**, especially if adjacent to a portal tract, are highly suggestive of bile-duct obstruction. However, because such infarcts are seen in only a minority of patients with obstructed ducts, the diagnosis must usually be established by other criteria.

As a result of these various forms of hepatocellular damage in biliary obstruction, and indeed in cholestasis generally, a certain amount of inflammatory infiltration of the parenchyma is commonly seen after a period of some weeks. This infiltration is usually mild and restricted to the cholestatic areas, unlike the inflammation of an acute hepatitis. When cholestasis resulting from duct obstruction is prolonged, especially in older patients, inflammation and liver-cell damage are occasionally severe enough to raise the alternative possibility of an acute hepatitis. It is then helpful to note that in bile-duct obstruction the liver-cell plates remain for the most part intact, whereas in hepatitis they become irregular as a result of cell loss, swelling and regeneration. Central–portal (zone 3) bridging necrosis is not a feature of biliary obstruction.

Within a few days or weeks of the onset of duct obstruction a characteristic triad of portal changes develops,[7] consisting of portal oedema and swelling (**Fig. 5.6**), infiltration by inflammatory cells and increased numbers of bile-duct profiles at the margins of the portal tracts (**Figs 5.7 and 5.8**). These marginal bile-duct structures are the most consistent finding in the portal tracts and are rarely absent.[7] They may originate from canals of Hering, periportal stem cells or other sources[8] and are an early response to the increased portal tract pressure due to obstruction, circulating mediators[9] and expression of developmental proteins such as Notch receptors and Jagged proteins.[10] The term **ductular reaction** refers to these proliferated bile ductules accompanied by inflammation and stromal changes at the edges of the

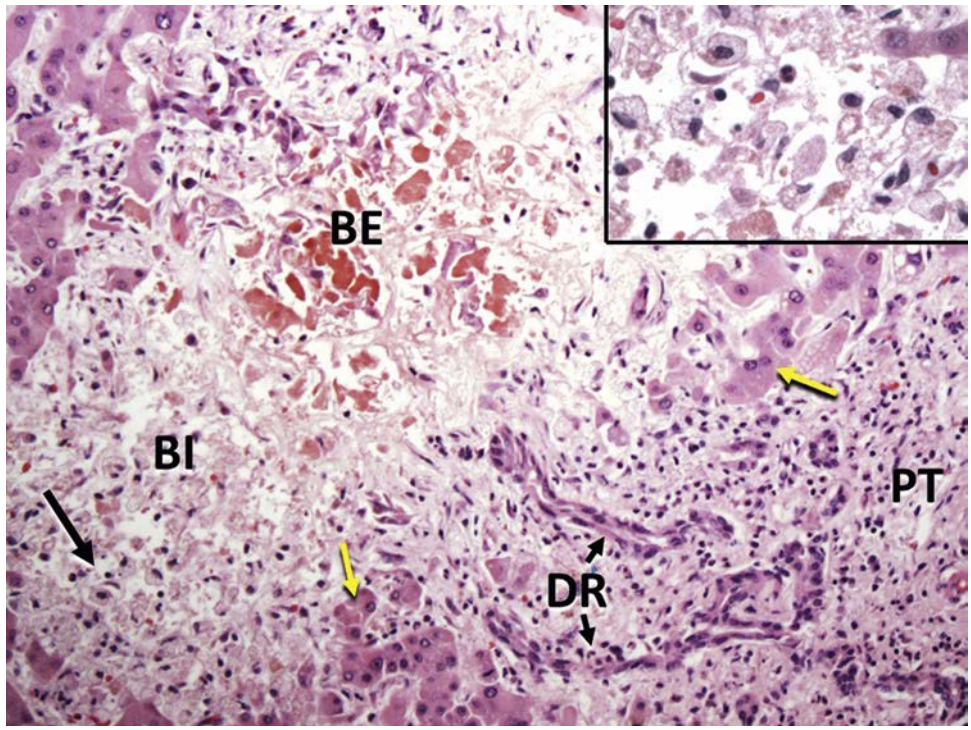

Fig. 5.5 Large bile duct obstruction with bile extravasate and bile infarct. Evidence of large duct obstruction is seen in the portal tract (PT) which is mildly oedematous and shows a prominent ductular reaction (DR) with inflammation. Bile has ruptured from the portal tract forming a bile extravasate (BE) with an adjacent bile infarct (BI). The infarcted hepatocytes are pale and slightly vacuolated with a faint brown, biliary cytoplasmic tinge and dark, pyknotic nuclei. Inset: Compare the pyknotic, contracted and basophilic nuclei of the infarcted hepatocytes with the normal-appearing hepatocytes of the periportal regions (yellow arrows). (Postmortem liver, H&E.)

portal tracts.[11,12] Usage of 'ductular reaction' is now preferable to 'bile ductular proliferation' or 'typical' and 'atypical' bile ductules, which embody considerable imprecision.[8] The ductular structures may be of normal calibre or dilated, but are often flattened with small or imperceptible lumens (**Fig. 5.8**) and variations in nuclear size, staining and location. These structures can be highlighted by immunostaining for cytokeratin 7 or 19 (**see Figs 5.25 and 5.26**). Surprisingly, bile is not usually seen within dilated ducts or ductules in uncomplicated obstruction; when it is present, sepsis should be suspected. The differentiation of the ductular reaction of biliary obstruction from that of chronic liver disease has already been discussed in **Chapter 4**.

Within the oedematous, swollen portal tracts, especially around proliferated bile ducts, an inflammatory infiltrate develops, mediated by the complex interactions of cytokines and cellular adhesion molecules (some produced by biliary epithelium itself[13a]) and proinflammatory agents such as endotoxin.[3] Neutrophils are prominent recruited there by the expression of chemokines such as interleukin-8, CXCL5 and others by the ductular cells.[13b,14] There may also be other cells, including lymphocytes and eosinophils. The presence of a few eosinophils is therefore not in itself sufficient evidence for a diagnosis of drug jaundice. As a result of the proliferative and inflammatory changes of bile-duct obstruction, the outlines of the portal tracts become irregular and the limiting plates of hepatocytes are disrupted to a variable extent. This disruption should be distinguished from interface hepatitis, in which the infiltrate is predominantly composed of lymphocytes and plasma cells, and in which the acute inflammatory changes of bile-duct obstruction are not seen.

In a few patients with bile-duct obstruction the portal changes are inconspicuous (**Fig. 5.6**) or even absent. Biliary obstruction should therefore be considered in the differential diagnosis of canalicular cholestasis without portal reaction (so-called pure or bland cholestasis). Conversely, portal changes resembling those of duct obstruction are occasionally

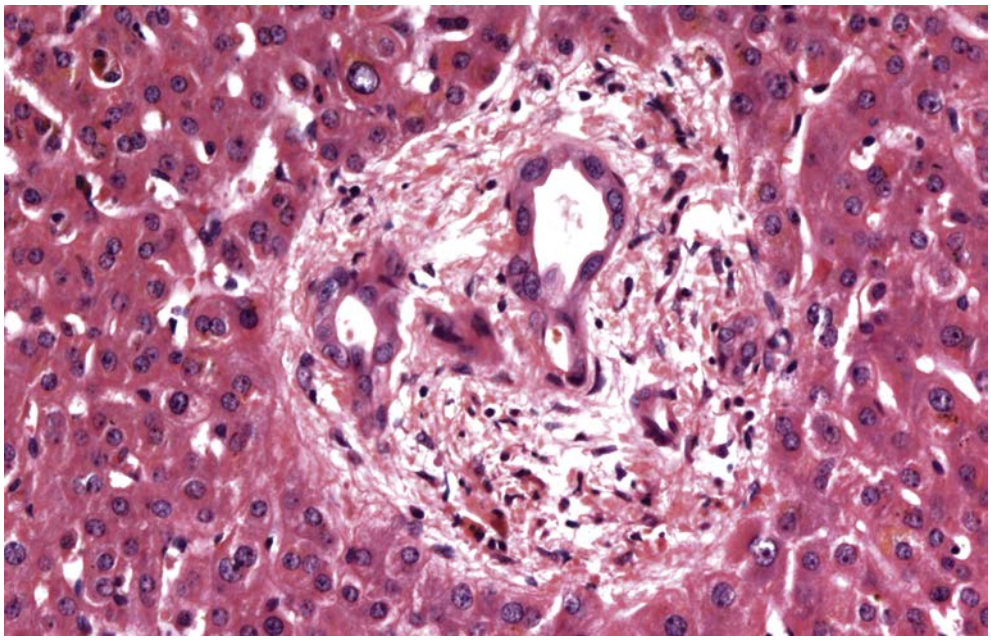

Fig. 5.6 Large bile-duct obstruction. The connective tissue of a small portal tract is oedematous. There is little inflammation in this example. (Wedge biopsy, H&E.)

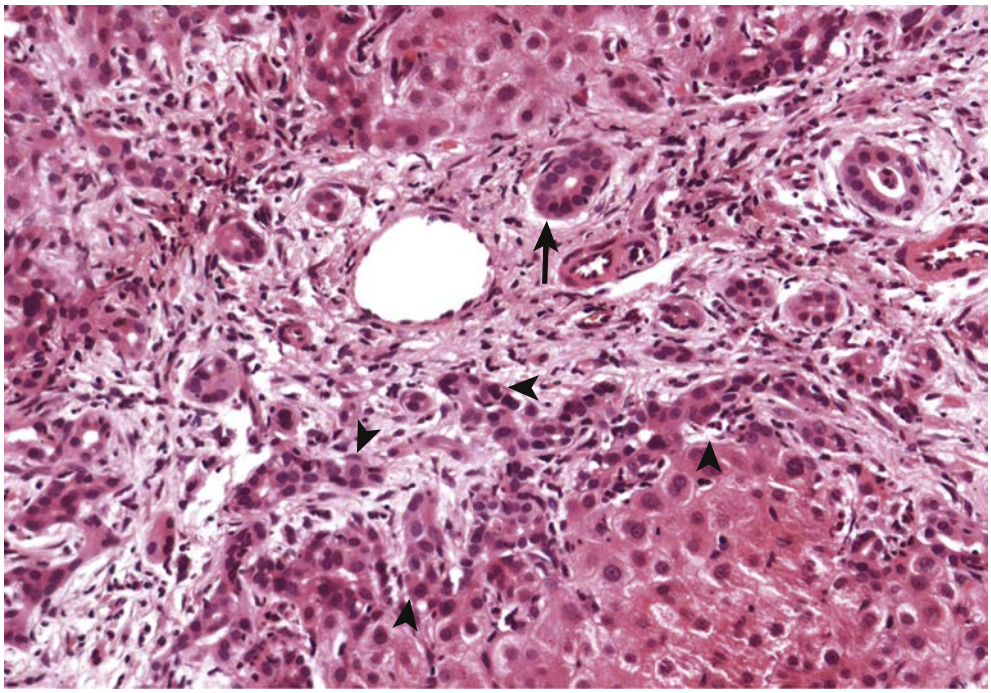

Fig. 5.7 Large bile-duct obstruction. A prominent ductular reaction (arrowheads) is present at the edge of an inflamed and oedematous portal tract. The original interlobular duct is marked by an arrow. (Needle biopsy, H&E.)

found in severe acute hepatitis, when the parenchymal alterations make the diagnosis clear. Sometimes similar portal changes are seen without cholestasis near space-occupying lesions such as metastases,[15] usually together with sinusoidal dilatation. Portal inflammation without cholestasis is also found in patients with disease affecting one or other part of the

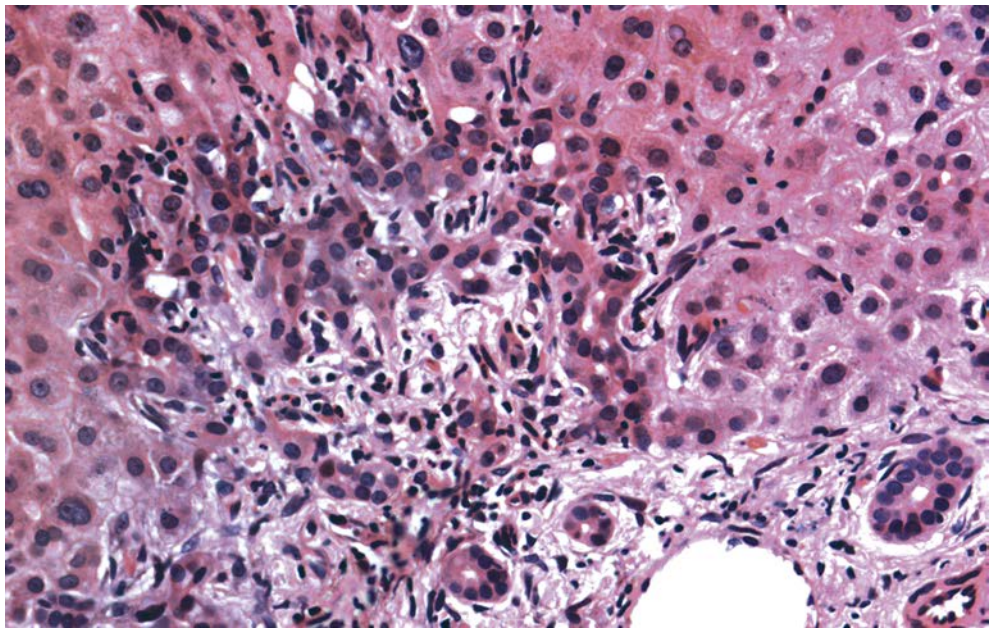

Fig. 5.8 Ductular reaction in large bile-duct obstruction. The upper left-hand portion of **Fig. 5.7** is shown at higher magnification. The irregular ductular structures at the edge of the portal tract show compressed, narrow lumens and an associated neutrophil infiltrate. (Needle biopsy, H&E.)

biliary tree but without current obstruction of the segment biopsied. It is seen in chronic pancreatitis[16] and in patients with acute cholecystitis or choledocholithiasis.[17] Biopsies showing only an increased number of well-differentiated bile ductules at the portal interface, unaccompanied by inflammation or stromal changes, have been noted in patients with idiopathic **isolated ductular hyperplasia**.[18] These patients have long-standing abnormalities in serum alanine aminotransferase and/or γ-glutamyl transferase, no proven biliary tract disease and an apparently good prognosis (although the cause of this reactive lesion is uncertain).

In a few instances of biliary obstruction, bile escapes from a duct into the connective tissue of a portal tract, giving rise to a *bile extravasate*. This leads to a phagocytic reaction, with or without foreign-body giant cells (**Fig. 5.9**). Bile extravasates, like large bile infarcts, are almost diagnostic of obstruction but are seen in only a minority of patients. If the extravasate extends beyond the confines of a portal tract into the adjacent parenchyma, the appearances at the periphery of the lesion are very like those of a bile infarct.

Chronic bile-duct obstruction and biliary cirrhosis

When bile-duct obstruction persists, the acute inflammatory reaction in the portal tracts is followed by increasing fibrosis. Production of fibrogenic cytokines by bile-duct epithelium contributes to this process.[19] Eventually the tracts are linked by broad fibrous septa. There is a variable degree of acute and chronic inflammatory infiltration; the chronic element is less striking than in PBC. In some patients the lesion appears to progress more by cholangitis than by obstruction, and cholestasis is therefore not always prominent or even present.

Interference with normal secretion of bile leads to several changes in hepatocytes adjacent to portal tracts and fibrous septa. The cells become swollen and separated by fibrous tissue or by hepatic progenitor cells, inflammatory cells[13b] and ductular structures (neocholangioles) derived from hepatocytes or bipotential stem cells.[20] Their cytoplasm is rarefied and may contain visible bile pigment, Mallory bodies, copper and copper-associated protein (**Fig. 5.10**). The last is seen in the form of fine red granules on haematoxylin and

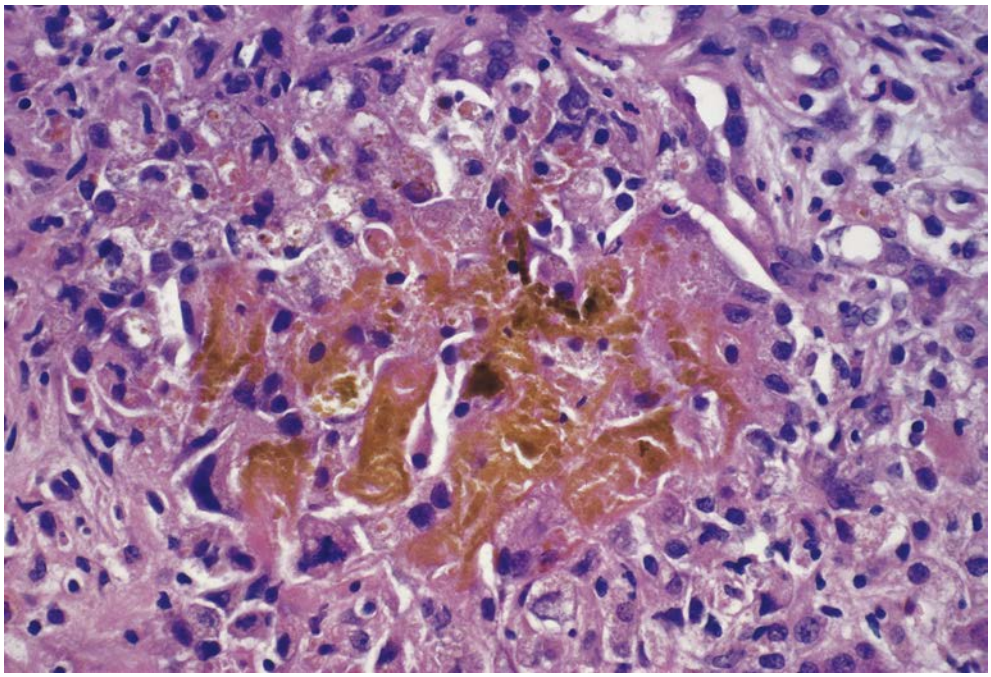

Fig. 5.9 Large bile-duct obstruction. Bile extravasate. Bile has escaped from a duct and has evoked a phagocytic reaction. (Needle biopsy, H&E.)

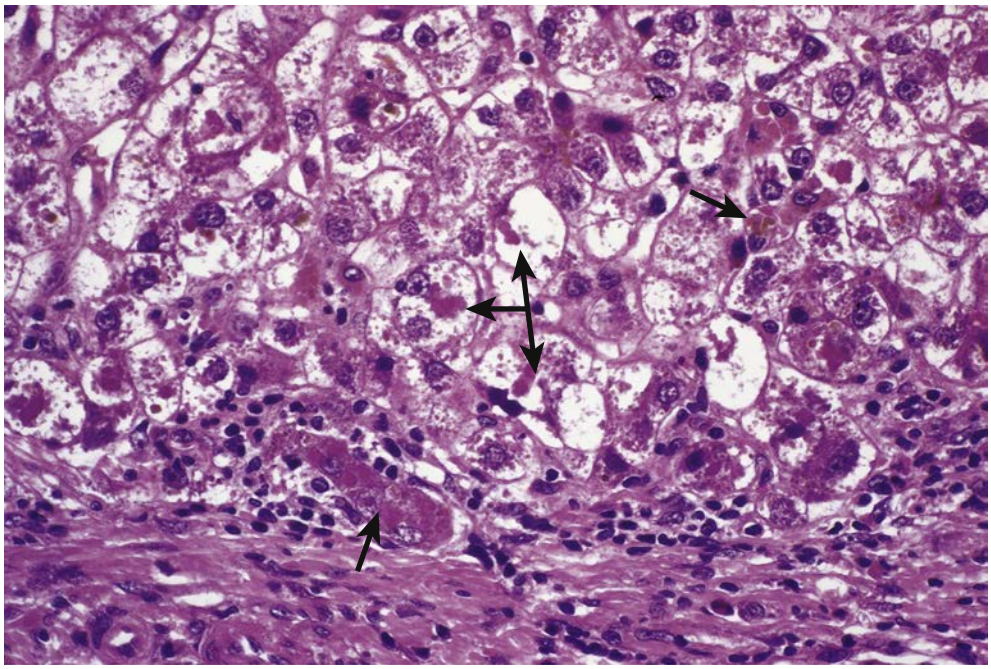

Fig. 5.10 Chronic cholestasis. Hepatocytes near a portal tract (at bottom) are swollen and pale-staining. Many contain Mallory bodies (lower arrow and triple arrow). Bile plugs are also seen (top arrow). (Wedge biopsy, H&E.)

eosin (H&E; **see** Fig. 5.27 inset), staining variably with diastase–PAS and strongly with orcein or Victoria blue. The combination of all these changes is known as **chronic cholestasis** or **cholate stasis** (**pseudoxanthomatous change**, **precholestasis**) on the basis that some of the alterations probably result from the accumulation of toxic bile salts.

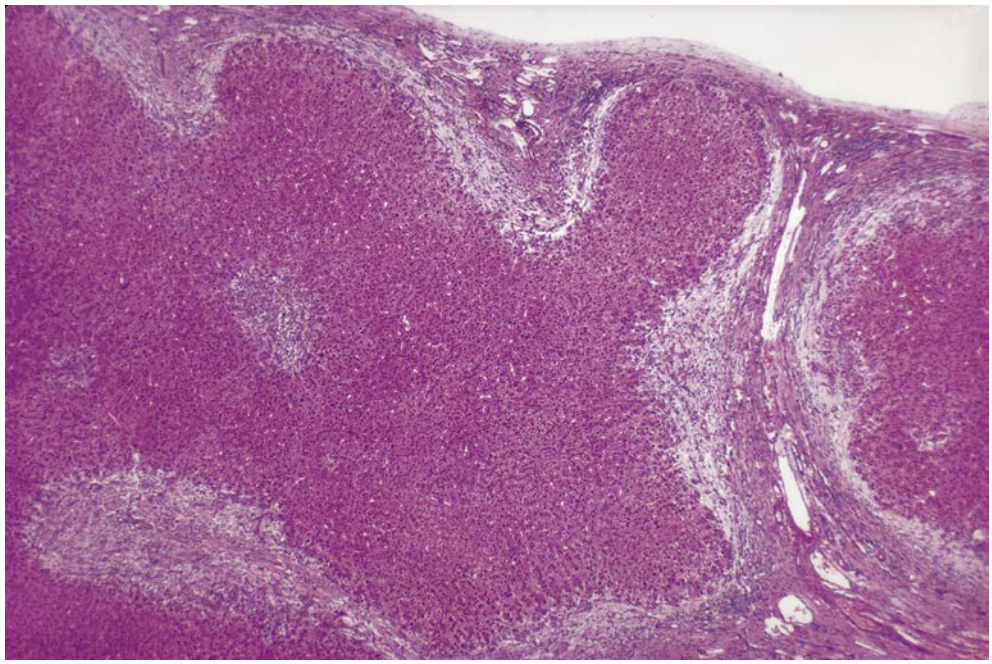

Fig. 5.11 Secondary biliary cirrhosis. Irregular nodules resemble pieces of a jigsaw puzzle. Note the narrow zone of oedema and ductular proliferation at the nodule margin. (Wedge biopsy, H&E.)

Canalicular cholestasis is sometimes seen between the affected hepatocytes. The hepatocellular changes, ductular proliferation and associated fibrosis in the periportal or periseptal region in effect produce an irregular interface with the parenchyma.[21]

The fibrous septa which eventually form in chronic biliary tract disease surround and outline groups of classical hepatic lobules, leaving the normal vascular relationships essentially intact. Islands of parenchyma with characteristic protruding studs resemble the pieces of a jigsaw puzzle or land masses on a map (**Fig. 5.11**). Spherical nodules are sparse at first, despite evidence of liver-cell hyperplasia in the form of thickened liver-cell plates, seen particularly in patients with associated portal hypertension.[22] An occasional rounded parenchymal island may merely represent a tangential section of a complex parenchymal mass such as the one shown in **Fig. 5.11**, rather than a true regeneration nodule of cirrhosis. This is especially common just deep to the liver capsule. A histological diagnosis of cirrhosis should therefore be made with caution, because at a fibrotic, precirrhotic stage considerable resolution can occasionally result if an obstruction is relieved.[23] Eventually, true **secondary biliary cirrhosis** develops, its biliary origin still evident from nodule shape and the regular, broad fibrous septa composed of loose collagen bundles with parallel arrangement (**Fig. 5.12**). A zone of oedema containing proliferated ductules is often diagnostically helpful and may be striking even at low magnification (the 'halo effect') (**Fig. 5.11**). Thus, many different structural characteristics make it possible to diagnose chronic biliary tract disease, even in the absence of cholestasis. Finally, however, an end-stage cirrhosis forms, no longer necessarily recognisable as biliary in origin.

Cholangitis: infection of the biliary tree

In biliary obstruction the inflammatory infiltrate around bile ducts in small portal tracts typically includes neutrophils. There is, therefore, cholangitis in a strictly histological sense, but this does not imply that there must be bacterial infection of the biliary tree or clinical ascending cholangitis. In the latter, neutrophils are more numerous and are found not only around ducts but also in their walls and lumens[24] (**Fig. 5.13**). Paradoxically, interlobular bile ducts

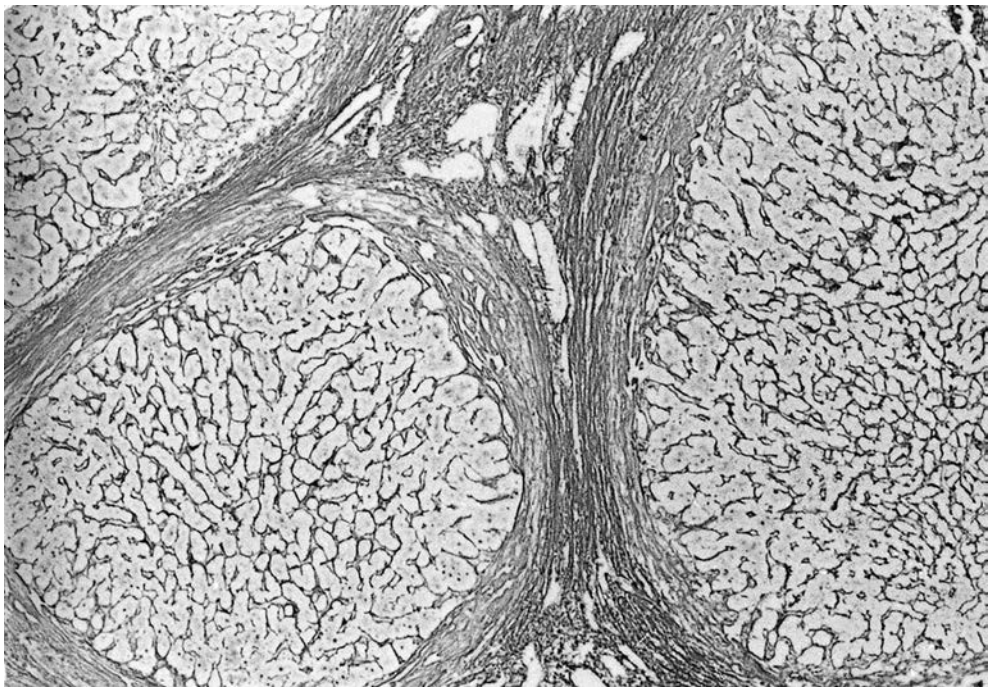

Fig. 5.12 Secondary biliary cirrhosis. Nodules are surrounded by loose bundles of parallel collagen fibres showing little compression. (Wedge biopsy, reticulin.)

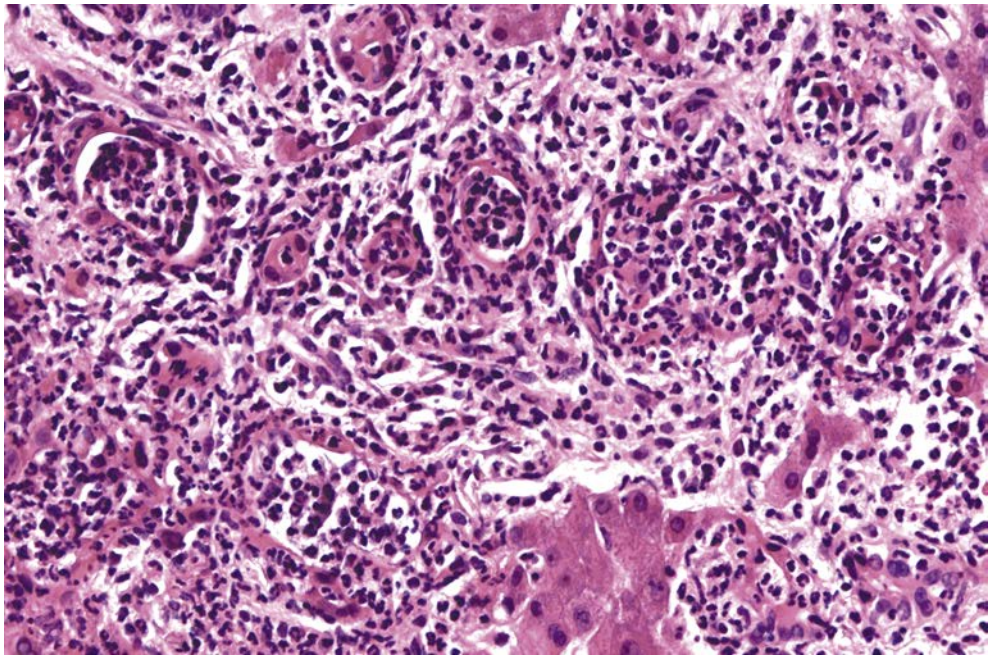

Fig. 5.13 Acute cholangitis. Many neutrophil leukocytes are seen in the walls and dilated lumens of the bile ducts, and in the surrounding connective tissue. (Wedge biopsy, H&E.)

are most affected, and larger ducts may appear histologically normal. The wall of a small duct may rupture, leading to abscess formation in the portal tract. Neutrophils are seen in the sinusoids and abscesses may form in the acini. Associated lesions include fibrin thrombi in portal-vein branches, pyelophlebitis and various degrees of parenchymal necrosis,[25] the last probably related to hypoperfusion of the parenchyma. Cholestasis is more often absent

than present. Causes of ascending cholangitis include cholecystitis and choledocholithiasis, strictures including those due to PSC, intrahepatic biliary stones,[26] AIDS cholangiopathy,[27,28] pancreatitis, neoplasia of the biliary tree and Caroli's disease. If cholangitis persists or recurs over a period of years, secondary biliary cirrhosis may develop. The histological features are then as described earlier in the section on bile-duct obstruction. Septicaemia uncommonly is associated with a particular form of histological cholangitis principally affecting the canals of Hering.[29] Affected ductules are dilated and filled with inspissated bile. Neutrophils accumulate around and sometimes within them. Larger ducts may be affected, as may the periportal parenchyma in which bile is seen in dilated bile canaliculi. These changes are easily confused with those of large bile-duct obstruction, but in obstruction the inspissated bile in the canals of Hering is not a feature unless there is concomitant sepsis. Sepsis more often gives rise to widespread canalicular cholestasis; the ductular cholestasis pattern (**see Fig. 15.12**) is seen in the minority of septic patients.[29] In toxic-shock syndrome the appearances of the small bile ducts can closely mimic ascending bacterial cholangitis.[30]

Primary sclerosing cholangitis

PSC is characterised by inflammation, strictures and saccular dilatations in the biliary tree. Typically found in adults with ulcerative colitis, it is also seen in neonates and children[31] and in the absence of inflammatory bowel disease and in several other clinicopathological settings (**Table 5.1**). In a few cases the latter is Crohn's disease rather than ulcerative colitis.[32] Any part of the biliary tree may be affected, and involvement of the gallbladder[33] and pancreas[34] has been reported. The gallbladder shows intramural lymphoplasmacytic infiltrates and lymphoid aggregates.[35] Patients do not necessarily have symptoms referable to the liver or abnormal liver function tests.[36] The disease may recur after liver transplantation.[37] Lesions similar to those of PSC have been found in patients given arterial infusion of the anticancer drug fluorodeoxyuridine and other[38] chemotherapeutic agents.[39] Obliteration or narrowing of hepatic arteries and portal-vein branches suggests that, in drug-related cases at least, the bile-duct damage may have an ischaemic origin.[40] Systemic vasculitis, liver transplantation-related hepatic artery thrombosis or chronic rejection vasculopathy and, rarely, septic shock[41] are other causes of ischaemic bile-duct injury[42] (ischaemic cholangiopathy). Similar bile-duct injury occurs after lengthy hospitalisations in intensive care units or trauma (conditions likely to be associated with hypotension/hypoperfusion-hypoxia) in 'secondary sclerosing cholangitis in critically ill patients'.[43–45] A paediatric variant form of PSC, autoimmune sclerosing cholangitis (ASC),[46,47] shows abundant clinicopathological autoimmune features and is discussed in further detail in Chapter 13. The pathogenesis of PSC is incompletely understood, but current genome-wide studies place this disease in an 'autoimmune' category, and there are additional potential contributions from gut microbiota, human leukocyte antigen (HLA) status, 'gut T cell-to-liver homing' and possible defects in bile-duct epithelium (cholangiocyte senescence).[48,49]

Final diagnosis of PSC normally rests on cholangiographic demonstration of the characteristic beading of bile ducts, but similar histological features, as described

Table 5.1 Variant forms of PSC.

Type of PSC	Population(s) involved/ comment
Primary sclerosing cholangitis • with inflammatory bowel disease • without inflammatory bowel disease	Adults, children
Small duct PSC	Adults + children Improved outcome*
PSC with autoimmune features (Overlap PSC/AIH syndrome)	Adults
Autoimmune sclerosing cholangitis (ASC)	Children
PSC with increased serum immunoglobulin G4	Adults Poorer prognosis*

*In comparison with classical PSC.

AIH, Autoimmune hepatitis; PSC, primary sclerosing cholangitis.

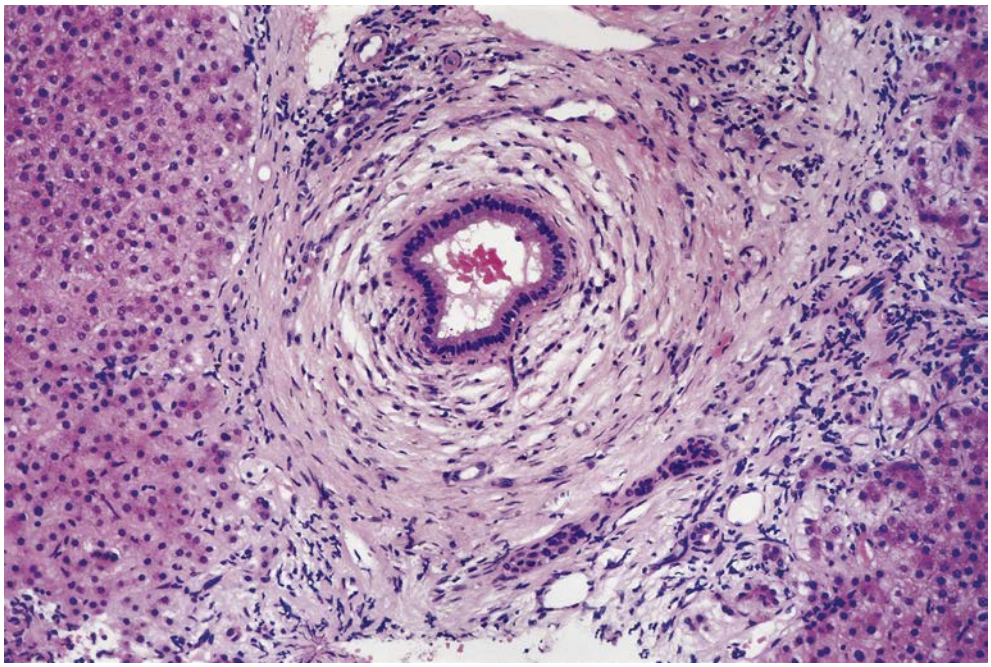

Fig. 5.14 Primary sclerosing cholangitis. A bile duct is surrounded by a cuff of oedematous, inflamed fibrous tissue with an 'onion-skin' appearance. (Needle biopsy, H&E.)

later, may be found in patients with normal cholangiograms. This can be explained on the basis of involvement of the smallest ducts, too small to be seen radiographically.[50] This **small-duct PSC** corresponds approximately to the now obsolete label of 'pericholangitis', when applied to patients without cholangiographic abnormalities. Large- and small-duct forms of the disease frequently coexist. Progression of small-duct PSC to large-duct PSC occurs in approximately 20% of cases, usually over a decade.[51]

The features seen on liver biopsy depend in part on the location of strictures in relation to the biopsy site. If, on the one hand, the biopsy is taken from a part of the liver unaffected by the primary disease but proximal to a stricture, then the changes, if any, will simply be those of bile-duct obstruction or cholangitis. The presence of chronic inflammation may lead to confusion with chronic hepatitis. If, on the other hand, the biopsy site is affected by the primary disease, there may be one or more features suggesting the diagnosis. These include periduct oedema and concentric fibrosis (**Fig. 5.14**), ductular proliferation, portal inflammation and atrophy or disappearance of the small ducts (**Fig. 5.15**). Loss of ducts is the most common finding in the smallest portal tracts, while periduct fibrosis is typical of medium-sized tracts.[52] Major bile ducts, as seen, for example, in explanted livers at transplantation, may be inflamed, ulcerated or dilated. They may also rupture, producing a perihilar **xanthogranulomatous cholangitis.**[53]

Loss of interlobular bile ducts from the smallest portal tracts can be assessed only in biopsy samples of adequate size, that is to say, containing several portal tracts. While interlobular ducts are not necessarily seen in all tracts because of the plane of section, arteries provide a useful guide: from 70%–80%[54] to 92%[55] of arteries are normally accompanied by a duct lying near the centre of a portal tract. If there is doubt, for instance because ducts are difficult to identify in an inflammatory infiltrate, immunostaining of duct-associated cytokeratins is helpful.[56] Suitable antibodies include AE-1 (Signet) and other antibodies against cytokeratins 7 and 19. In the presence of a ductular reaction, identification and counting of interlobular bile ducts are sometimes difficult.

The concentric fibrosis around medium-sized ducts is not entirely diagnostic because it is occasionally found in other forms of biliary disease such as hepatolithiasis.[57] It is, however, a very helpful finding. The lamellar pattern of the fibrosis gives an 'onion-skin'

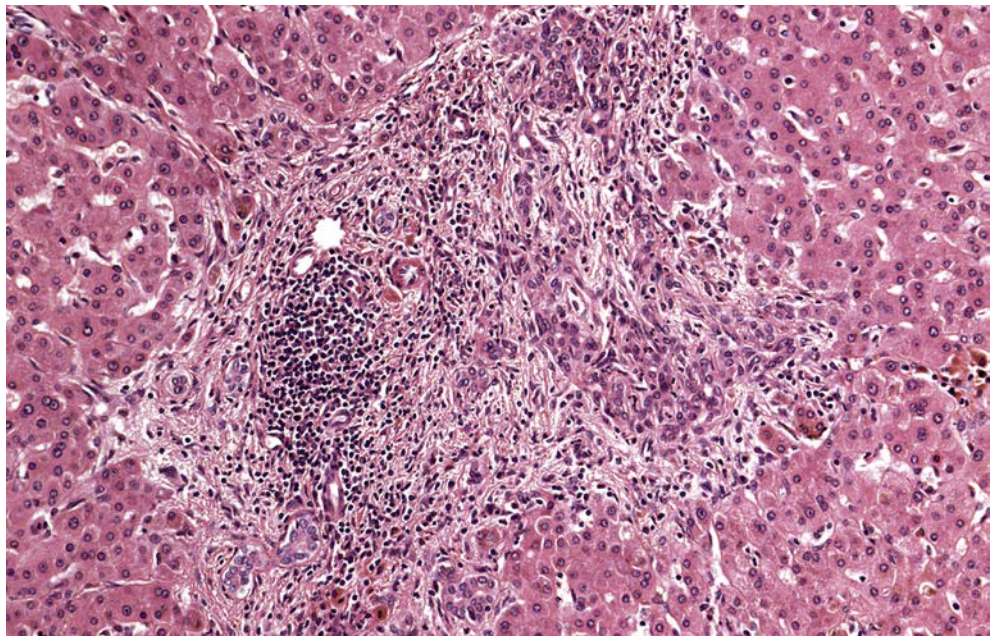

Fig. 5.15 Primary sclerosing cholangitis. The inflamed portal tract lacks a bile duct. An aggregate of lymphocytes to the left of a small hepatic arteriole at the centre of the field is likely the former site of the duct. Inflammation extends into the adjacent parenchyma and there is interface hepatitis to the right of the portal tract. (Wedge biopsy, H&E.)

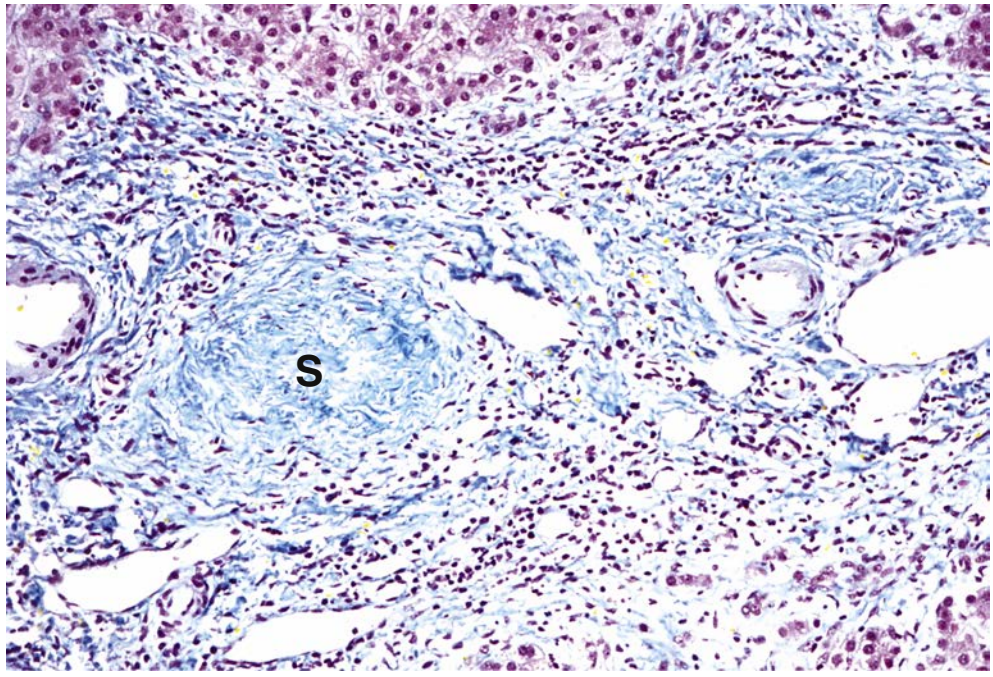

Fig. 5.16 Primary sclerosing cholangitis. The bile duct in a large portal tract has been replaced by a fibrous scar (S). (Wedge biopsy, H&E.)

appearance. The cuff of connective tissue around the duct may be oedematous and pale-staining or sclerotic, depending on the stage of the process. Inflammatory cells are seen in small numbers lying between the layers of collagen. The duct epithelium may show various degrees of atrophy, and sometimes disappears entirely, leaving a characteristic rounded fibro-obliterative scar[58] (**Fig. 5.16**). Staining with diastase–PAS often reveals irregular or regular thickening of the basement membrane material around both scarred and unscarred

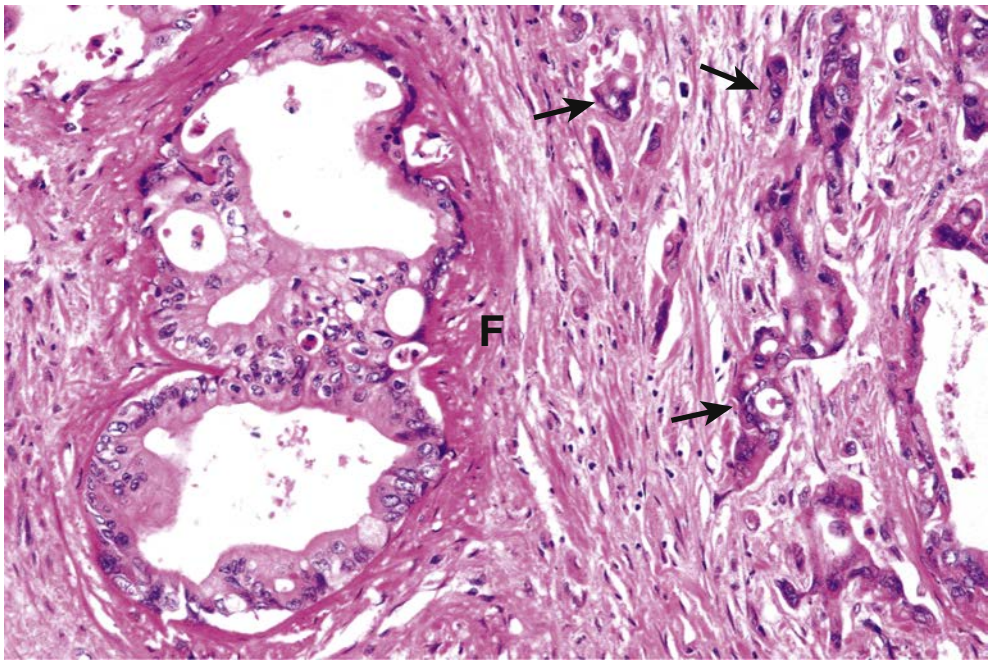

Fig. 5.17 Cholangiocarcinoma in primary sclerosing cholangitis. Carcinoma has developed in the bile duct at left, still surrounded by periduct fibrosis (F). Invasive glands (arrows) are seen in the adjacent stroma. (Explanted liver, H&E.)

ducts.[59] In long-standing or severe cases, portal fibrosis gradually increases, fibrous septa form and secondary biliary cirrhosis may develop. In some patients, by contrast, the lesions remain mild and clinically insignificant for many years.[32,36] Portal tract fibrogenesis in sclerosing cholangitis and in PBC is in part attributed to an increased number of intrahepatic mast cells compared with other chronic liver diseases.[60] In fact, systemic mastocytosis has been associated with cholestasis[61] and a case of PSC.[62]

Parenchymal changes in PSC are usually less striking than the portal ones. Cholestasis may be seen as a result of large-duct obstruction or small-duct loss. In the later stages, the cholestasis is typically of the chronic type, with accumulation of copper and copper-associated protein. Liver cells may undergo hyperplasia, indicated by thickening of cell plates. Extension of portal tract lymphoplasmacytic infiltrates into the periportal parenchyma (interface hepatitis) is common but not as a rule severe (**Fig. 5.15**). However, more severe interface hepatitis may be seen in patients with an unfavourable clinical course[32] or in an overlap syndrome with AIH. In children, an autoantibody-positive, immunosuppression-sensitive cholangiopathy designated as the entity ASC[46,63] shows a chameleon-like pattern alternating between AIH and PSC both conditions in its evolution over time, as discussed in Chapter 13.

Histological assessment of liver biopsies in patients with an established diagnosis of PSC is important for prognosis. Ludwig and colleagues[50,64,65] have proposed a histological staging system based on essential and non-essential features. The stages correspond approximately to those of PBC: they are respectively designated portal, periportal, septal and cirrhotic. The Ishak and Nakanuma scoring systems for chronic hepatitis and primary biliary cholangitis, respectively, can also effectively be utilized for prognostication in PSC.[66,67]

There is an increased risk of carcinoma of the biliary tree in patients with sclerosing cholangitis[68] (**Fig. 5.17**) who may also have dysplasia of interlobular and septal bile ducts[69] (**Fig. 5.18**) and gallbladder,[70] including papillary bile-duct dysplastic lesions.[71] Cholangiocarcinoma-associated cytogenetic abnormalities such as polysomy (gains in chromosomes 3, 7 and 17) and loss of the CDKN2A gene for P16 at the 9p21 locus can be demonstrated in cytological and tissue specimens from bile ducts with dysplasia in PSC.[72] The same risk of carcinoma does not appear to apply to the small-duct form of PSC.[73]

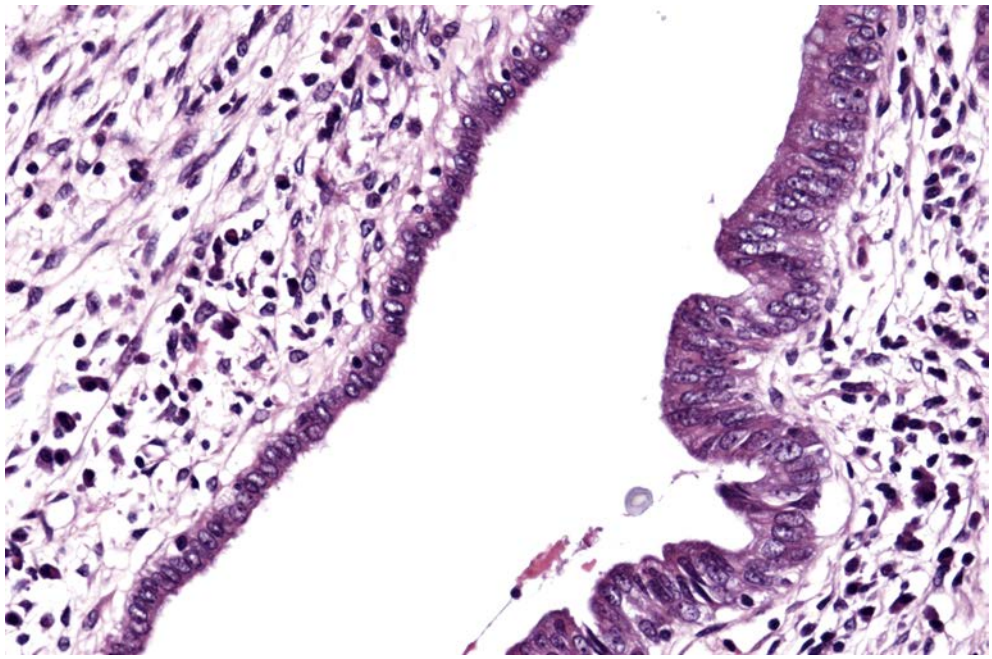

Fig. 5.18 Bile-duct dysplasia in primary sclerosing cholangitis. The epithelium of the right portion of the bile duct is crowded and adenomatous and shows nuclear atypia. (Operative specimen, H&E.)

The main **differential diagnosis** of PSC is from chronic hepatitis, PBC and other forms of chronic biliary tract disease. In **chronic hepatitis** bile-duct numbers are normal, periduct fibrosis is not seen and cholestasis is very uncommon. Stains for copper and copper-associated protein are negative or near-negative unless cirrhosis has developed.[74] **PBC** closely resembles PSC in its later stages, and firm diagnosis usually requires cholangiography and testing for antimitochondrial antibodies (AMAs). However, the typical granulomatous cholangitis of PBC is not a feature of sclerosing cholangitis, although granulomas are very occasionally found in the liver.[75] Substantial chronic inflammation of portal tracts with or without lymphoid follicles favours PBC. Conversely, fibrous obliteration of ducts is much more characteristic of sclerosing cholangitis, and there is often dense portal fibrosis with relatively little inflammation. The main difference from **other chronic biliary diseases** is the loss of ducts and interface hepatitis. There is occasionally confusion between the focal duct dilatations of **Caroli's disease** and the cholangiectases which are typically seen in the large- and medium-sized bile ducts in PSC.[68,76] **Immunoglobulin G4 (IgG4)-associated cholangitis** (IAC), also termed **IgG4-related sclerosing cholangitis** (IRSC),[77–81a,81b] a steroid-sensitive, autoimmune cholangiopathy, also needs consideration because it causes histological changes similar to PSC[82,83] and may raise the clinical question of cholangiocarcinoma.[84] This condition is part of the spectrum of autoimmune diseases featuring IgG4+ plasma cells and sclerosis (IgG4-related disease[78]) which also includes autoimmune pancreatitis and certain cases of inflammatory pseudotumour.[85] Like PSC, the large ducts in IAC are surrounded by dense lymphoplasmacytic infiltrates (often with lymphoid aggregates and follicles), but in IAC the infiltrates extend deeply into the soft tissues surrounding the ducts with entrapment of nerves and obliterative phlebitis of large-calibre veins The basic histological features of IgG4-related diseases should be present, including dense lymphoplasmacytic infiltrate, storiform-type fibrosis and obliterative phlebitis[86] (**Fig. 5.19**). In contrast to other chronic biliary diseases where IgG4+ plasma cells are absent or sparse, IAC shows numerous IgG4+ plasma cells on immunostaining (>50 per high-power field[86]). A subgroup of more clinically aggressive PSC cases may also show such increased IgG4+ plasma cells.[87] Liver biopsies in IAC show portal tract infiltrates of

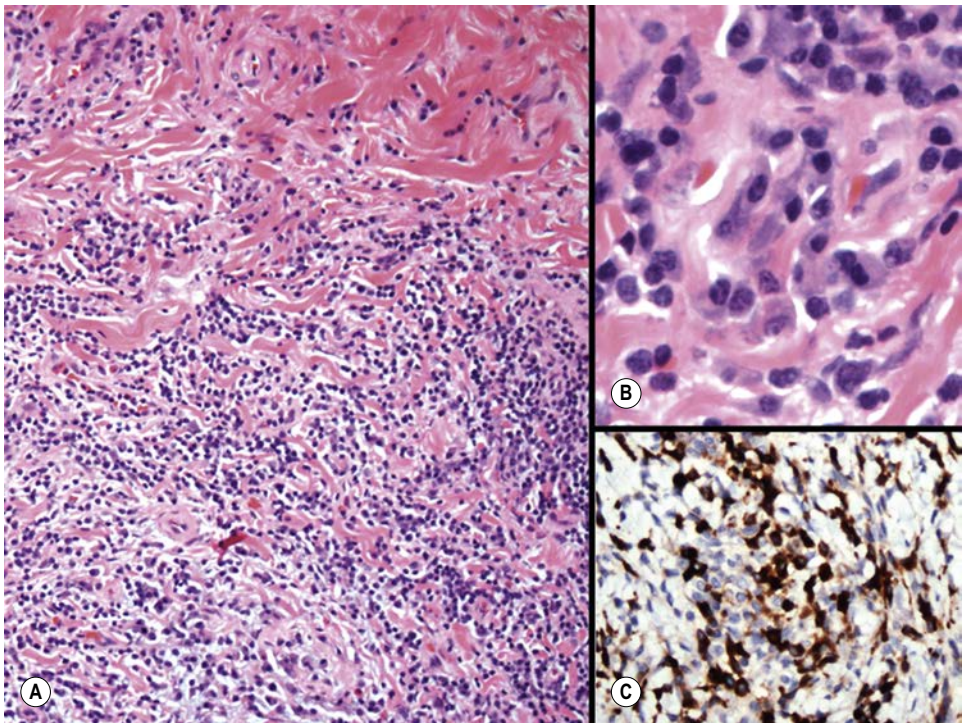

Fig. 5.19 Immunoglobulin G4 (IgG4)-associated cholangitis. A: An obstructing mass at the liver hilum leading to biliary obstruction was suspicious for malignancy, but wedge resection instead shows markedly inflamed connective tissue with dense collagen bundles arranged in a whirled 'storiform' pattern. **B:** Numerous plasma cells are present in the infiltrates which also featured lymphocytes and scattered eosinophils. **C:** Immunostain for IgG4 shows diffuse, strong positivity of the plasma cells. (Wedge resection; **A&B**: H&E; **C**: specific immunoperoxidase.)

lymphocytes, eosinophils and increased IgG4+ plasma cells (>10 per high-power field).[86] The inflammation is accentuated around portal vein branches, and portal connective tissue is widened by storiform fibroinflammatory nodules.[88]

Primary biliary cholangitis (formerly primary biliary cirrhosis)

Primary biliary cirrhosis (PBC) was officially re-named *primary biliary cholangitis* in 2015 by international consensus.[89] PBC is generally regarded as an autoimmune disease and is characterised by a chronic non-suppurative destructive cholangitis which can eventually lead to cirrhosis.[90] However, because in most affected individuals the disease is slow—even decades—in its evolution to cirrhosis, even shortly after the original term was coined,[91] Professor Dame Sheila Sherlock advocated for better terminology.[92] PBC typically presents in middle life, but may also be found in the elderly, younger adults and uncommonly in adolescents.[93] Women are about 10 times more likely to be affected than men. In symptomatic patients the onset is insidious, with itching as the most common presenting symptom. Jaundice and histological cholestasis are usually absent in the early years of the disease. Characteristic findings on investigation of both symptomatic and asymptomatic patients include raised level serum alkaline phosphatase and the presence of AMAs. The antibodies are specifically the M2 type, directed against inner mitochondrial membrane autoantigens, which are members of the 2-oxo-acid dehydrogenase complex (2-OADC) of enzymes.[94] The most common of these antibodies reacts with the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2). AMAs can be detected in more than 90% of patients. Serum IgM values are typically elevated.[95] Current hypotheses suggest that expression of 2-OADC antigens on bile-duct epithelium together with appropriate class II histocompatibility antigens, production of AMAs and T-lymphocyte response[96] mediate the bile-duct damage in PBC.[97] Cross-reactivity of human AMAs with bacterial antigens on *Escherichia coli* and other

organisms that may infect patients with PBC has been suggested in the 'molecular mimicry' hypothesis.[98] Dysregulated interleukin-12 signalling[99] and increased circulating and intrahepatic T follicular helper cells ($CD4^+$) involved in B-cell activation[100,101] have also been described. Environmental exposures to xenobiotics may potentially alter the lipoyl moiety of PDC-E2 and cause loss of immune tolerance.[102–104]

PBC is associated with a wide range of other conditions, many of them regarded as autoimmune in origin. The most common association is with the sicca complex of dry eyes and mouth.[105] Others include scleroderma, thyroiditis, rheumatoid arthritis, membranous glomerulonephritis and coeliac disease. Interstitial lung disease is present in some individuals with PBC and lung biopsies show many histological features in common with the liver disease, including poorly formed granulomas, eosinophil infiltrates and lymphoplasmacytic interstitial infiltrates.[106]

Box 5.2 Stages of primary biliary cholangitis

1. The florid duct lesion; portal hepatitis
2. Ductular reaction and periportal hepatitis
3. Scarring; bridging necrosis, septal fibrosis
4. Cirrhosis

Liver biopsy plays an important part in diagnosis throughout the often long course of the disease. Four histological stages have been described[64,90,107] (**Box 5.2**). These are not always easy to determine in needle biopsies, partly because the lesions of PBC are unevenly distributed within the liver and partly because the stages overlap. For example, stage 1 bile-duct lesions and granulomas are sometimes seen in an established cirrhosis. From a practical point of view, however, the pathologist is usually able to decide whether the disease appears to be still in stage 1, with lesions more or less restricted to enlarged portal tracts, or whether it has extended to a significant degree into the adjacent parenchyma, with consequent alteration of acinar structure (the progressive lesion; stages 2, 3 or 4). This is of some clinical importance, because stage 1 often lasts for many years and the prognosis is therefore relatively favourable, especially in patients without symptoms referable to the liver. Having established in other patients that the disease has progressed beyond stage 1, the pathologist may also be able to determine with reasonable confidence that cirrhosis has developed. The patient is then at increased risk for hepatocellular carcinoma,[108,109] sometimes preceded by macroregenerative nodule formation.[110] However, other risk factors such as hepatitis C virus infection must be considered in patients with PBC who develop carcinoma.[111] Because different sets of differential diagnoses should be considered for the portal and the progressive lesion, these are considered separately in the following section.

The portal lesion of primary biliary cholangitis

The bile-duct damage characteristic of early PBC mainly affects the septal and larger interlobular ducts, while the smaller interlobular ducts remain intact until later. The epithelium of the affected ducts becomes irregular and is infiltrated with lymphocytes. The basement membrane becomes disrupted, and the duct may rupture (**Fig. 5.20**). An inflammatory infiltrate is seen around or to one side of the duct. The denser parts of this infiltrate are mainly composed of lymphocytes, which may form aggregates or follicles with germinal centres (**Fig. 5.21**). Elsewhere there is a mixture of plasma cells (often abundant), eosinophils and neutrophils. The eosinophils contribute to bile-duct damage, granuloma formation and other aspects of the inflammatory response by releasing mediators which are located within their granules.[112] The biochemical and/or histological improvement seen in certain patients treated with ursodeoxycholic acid appears to be attributable in part to inhibition of eosinophil degranulation.[113] Of the various histological features of the disease, interface hepatitis appears to be the most resistant to improvement with ursodeoxycholic acid.[114]

In line with the presence of elevated serum IgM in individuals with PBC is the predominance of IgM-positive plasma cells in the portal tract plasmacytic infiltrates.[115] Specific IgM immunostaining can therefore be diagnostically useful (and contrasts with

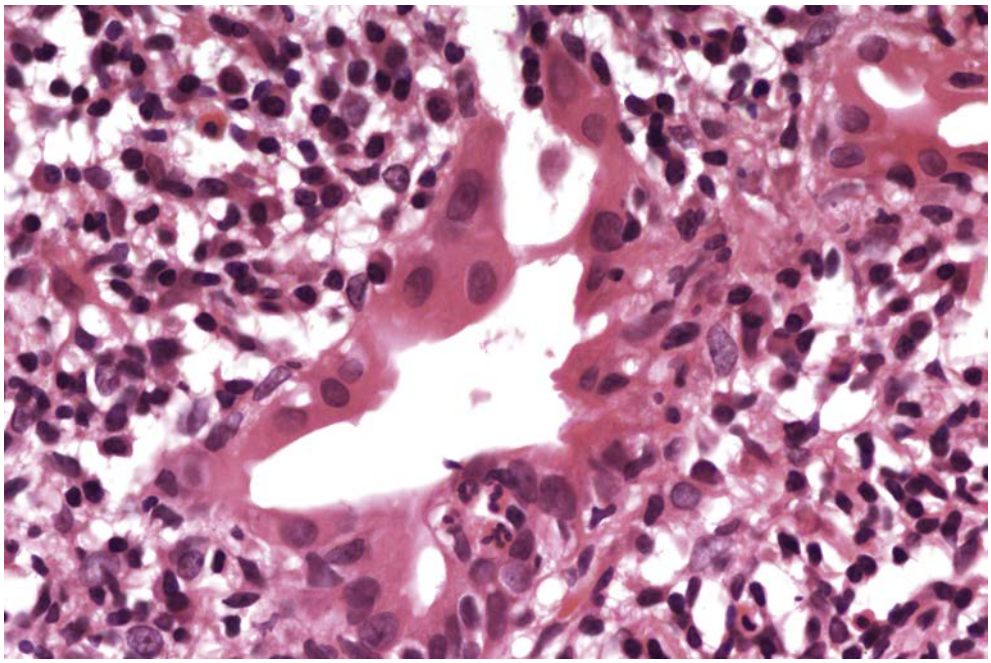

Fig. 5.20 Primary biliary cholangitis. A damaged large interlobular bile duct shows an irregular configuration, partly attenuated epithelium and intraepithelial inflammatory cells. The surrounding infiltrate is rich in lymphocytes and plasma cells. (Wedge biopsy, H&E.)

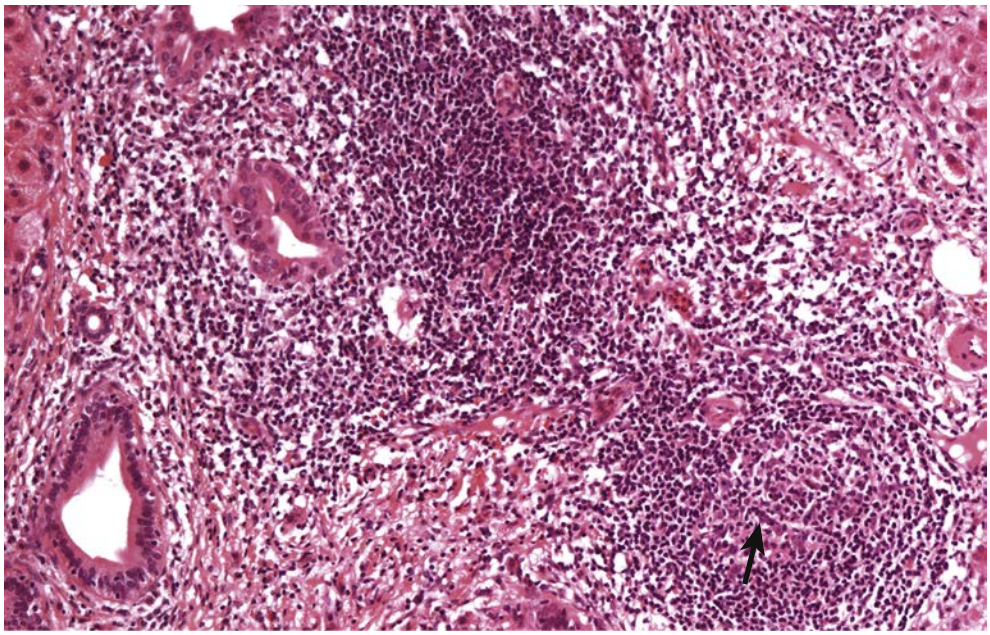

Fig. 5.21 Primary biliary cholangitis. A lymphoid aggregate and a follicle with a germinal centre (arrow) are seen near an inflamed duct with stratified epithelium. (Wedge biopsy, H&E.)

the IgG-predominant plasma cells seen in AIH[115,116]). CD1a-positive Langerhans cells may be increased within the predominantly lymphocytic intraepithelial bile-duct infiltrates.[117]

Granulomas are present in many patients, although they are not necessarily seen in small biopsies; their absence does not therefore exclude the diagnosis. They take a variety of forms,[118] ranging from well-defined granulomas like those of sarcoidosis or tuberculosis (**Fig. 5.22**) to small focal collections of histiocytoid cells. Alternatively, there may be a substantial component

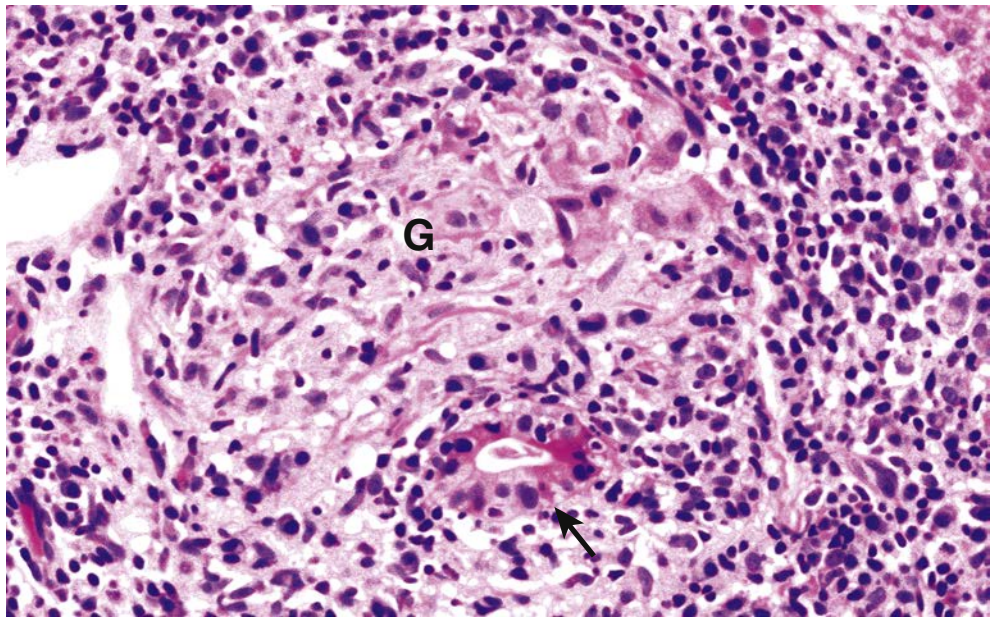

Fig. 5.22 Primary biliary cholangitis. A well-formed epithelioid-cell granuloma (G) has formed near a damaged bile duct (arrow). The background infiltrate contains many lymphocytes, plasma cells and scattered eosinophils. (Needle biopsy, H&E.)

of histiocytes or epithelioid cells within the inflammatory infiltrate, without formation of identifiable localised granulomas. A few intra-acinar granulomas may also be present (usually small intrasinusoidal clusters of histiocytes rather than well-formed granulomas), but large numbers should suggest the diagnosis of other granulomatous diseases (**see Ch. 15**).

Not all liver biopsies from patients in this stage of the disease show the typical bile-duct lesions, so that a firm histological diagnosis cannot always be made. Small portal tracts may merely show 'non-specific' portal inflammation, in which case step sections may make the true diagnosis clear by revealing bile-duct lesions or granulomas. In a small number of patients with the **premature ductopenic variant** of PBC,[119] widespread bile-duct destruction and loss are accelerated at an early stage before the development of fibrosis or cirrhosis, with worse pruritus and clinical evidence of chronic cholestasis than would be anticipated.

Although in the first stage of PBC the lesions are by definition mainly portal, slight disruption of the limiting plate is common. Sinusoids may be infiltrated by lymphocytes, Kupffer cells are prominent, and there may be focal necrosis[120] and thickening of liver-cell plates. Nodular regenerative hyperplasia, best recognised in reticulin preparations, is common even at this stage[121,122] and, together with portal vein narrowing,[123] helps to explain the portal hypertension which frequently precedes the development of significant fibrosis or cirrhosis. Foci of small hepatocytes with basophilic cytoplasm and hyperchromatic nuclei (small-cell dysplasia) or hepatocytes with enlarged, pleomorphic nuclei (large-cell dysplasia) are occasionally found.[124]

Canalicular cholestasis is unusual in early PBC unless there is a complicating factor such as steroid-induced jaundice. Cholestasis of the chronic type (cholate stasis) does not develop until later, although small amounts of copper-associated protein are occasionally seen in periportal hepatocytes.

The **differential diagnosis of early PBC** includes other causes of portal inflammation and of bile-duct damage. The differentiation from **PSC** was discussed earlier. In PSC duct atrophy and fibrosis predominate and granulomas are seen only rarely.[75] **Drug injury** occasionally leads to bile-duct damage, but the ducts affected are smaller than those in early PBC; other parenchymal changes (fat, hepatocyte ballooning and apoptosis) are often present, and the lesion is seen in the clinical context of an acutely jaundiced patient. Amoxicillin–clavulanic acid hepatotoxicity is an example of PBC-like, eosinophil-rich bile-duct damage.[125] Bile ducts

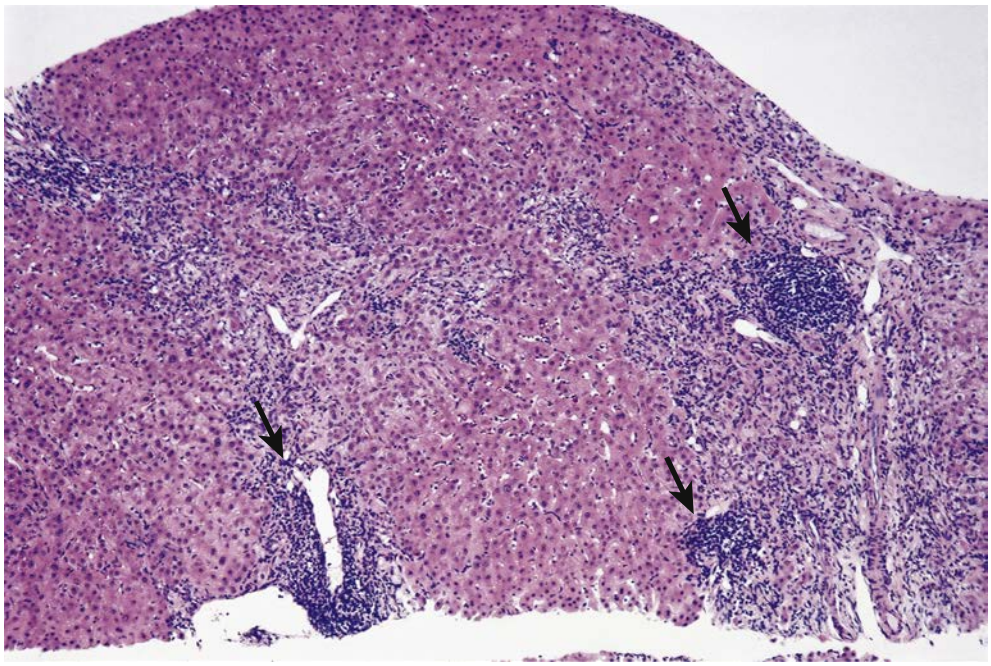

Fig. 5.23 Primary biliary cholangitis. Aggregates of lymphocytes (arrows) mark the former sites of bile ducts in this inflamed, fibrotic liver. The picture is very typical of the progressive phase of the disease. (Needle biopsy, H&E.)

are often abnormal in acute and chronic **viral hepatitis**, especially hepatitis C.[126] In hepatitis the epithelium of the affected ducts may be abnormal in only part of its circumference (**see Fig. 6.8**), and is typically stratified and vacuolated.[127] The surrounding infiltrate is almost entirely composed of lymphocytes, with few plasma cells or segmented leukocytes and no granulomas. Large numbers of eosinophils, sometimes seen in PBC,[128] are rare. In doubtful cases the clinical context and laboratory investigations usually make the diagnosis clear. Because in viral hepatitis the duct damage is focal and does not lead to extensive duct loss, the clinical and biochemical picture is not necessarily cholestatic. Other causes of bile-duct damage include **AIH,**[129] **bile-duct obstruction with suppuration**, **graft-versus-host disease** and **rejection of a grafted liver**. The last two situations are discussed in **Chapter 16**. Two rare causes of bile-duct damage associated with granulomas are **fascioliasis** and **sarcoidosis**, but in general this association strongly supports a diagnosis of PBC.

The progressive lesion of primary biliary cirrhosis

The disease now extends beyond the confines of the portal tracts, and there is increasing fibrosis and alteration of acinar architecture. Bile-duct damage is less dramatic and granulomas are fewer, but there is a progressive fall in duct numbers. Duct numbers are best assessed in relation to arteries,[54] as already discussed in relation to PSC. The sites of former ducts are marked by aggregates of lymphocytes (**Fig. 5.23**). These sometimes show compression artefact, with rupture of lymphocyte nuclei. The inflammatory reaction may also obliterate periductal capillaries.[130]

The portal tracts expand progressively as the inflammatory process begins to extend from them into the adjacent parenchyma. At this time two apparently separate processes affect the future course of the disease. The first comprises a combination of biliary and cholestatic features, probably related to bile-duct loss, while the second closely resembles the interface hepatitis of chronic hepatitis.[21,131] The earliest and often most obvious biliary feature is a ductular reaction (**Fig. 5.24**). For a time this allows bile to drain from the parenchyma into the main ducts despite destruction of the medium-sized ducts.[132] It is almost

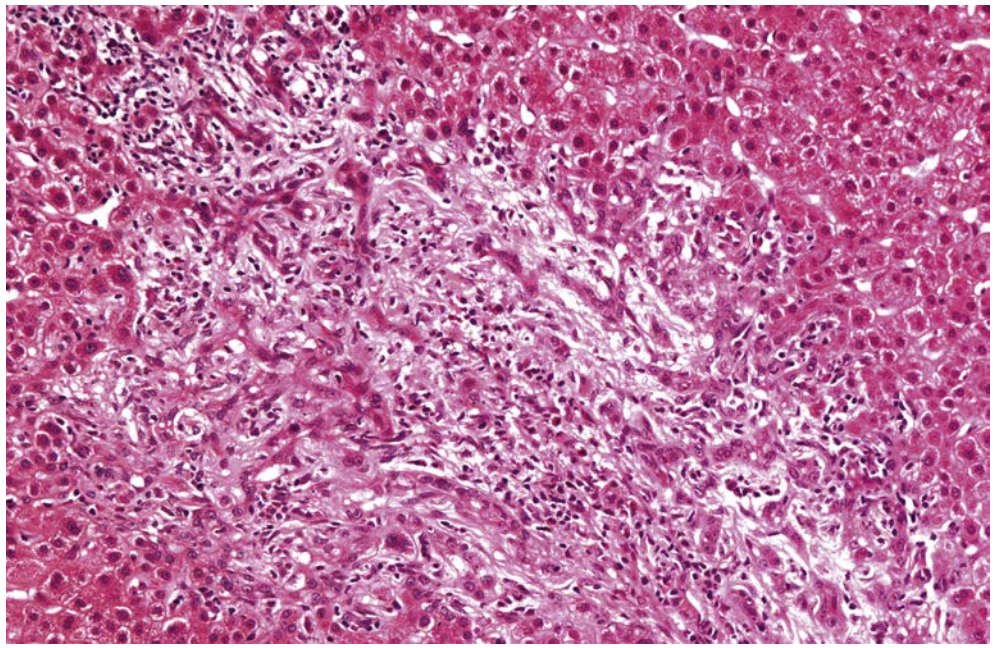

Fig. 5.24 Primary biliary cholangitis. A widened portal tract shows chronic inflammation and a ductular reaction but no native bile duct. The margins of the tract are blurred by fibrosis and interface hepatitis. (Wedge biopsy, H&E.)

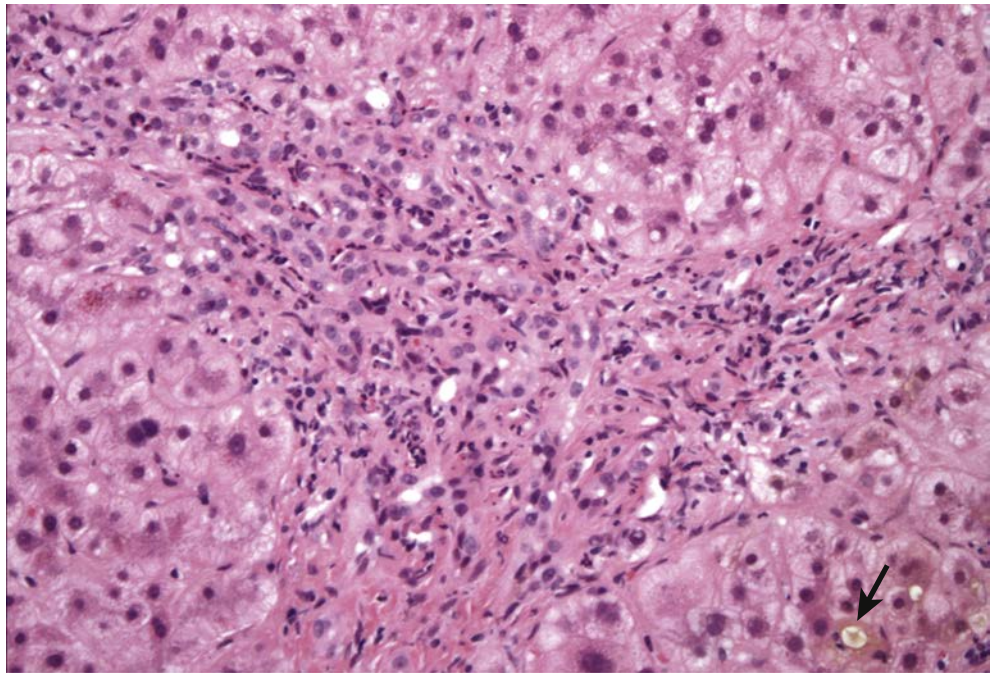

Fig. 5.25 Stage 3 primary biliary cholangitis. The portal tract is chronically inflamed and fibrotic, without a readily identified bile duct. A ductular reaction is present, but obscured by the inflammation and fibrosis. Cholestasis is present at the periphery of the lobule (arrow). (Needle biopsy, H&E.)

always associated with an infiltrate of neutrophils, so that it needs to be distinguished from the duct tortuosity and inflammation of mechanical bile-duct obstruction. The ductular reaction of PBC, and indeed of PSC, is often focal, representing a system of bypass channels in relation to a local interruption of bile flow through the duct system. If the ductular structures are partly obscured by inflammation and fibrosis (Fig. 5.25), they can be highlighted by immunostaining for cytokeratin 7 or 19 (Fig. 5.26).

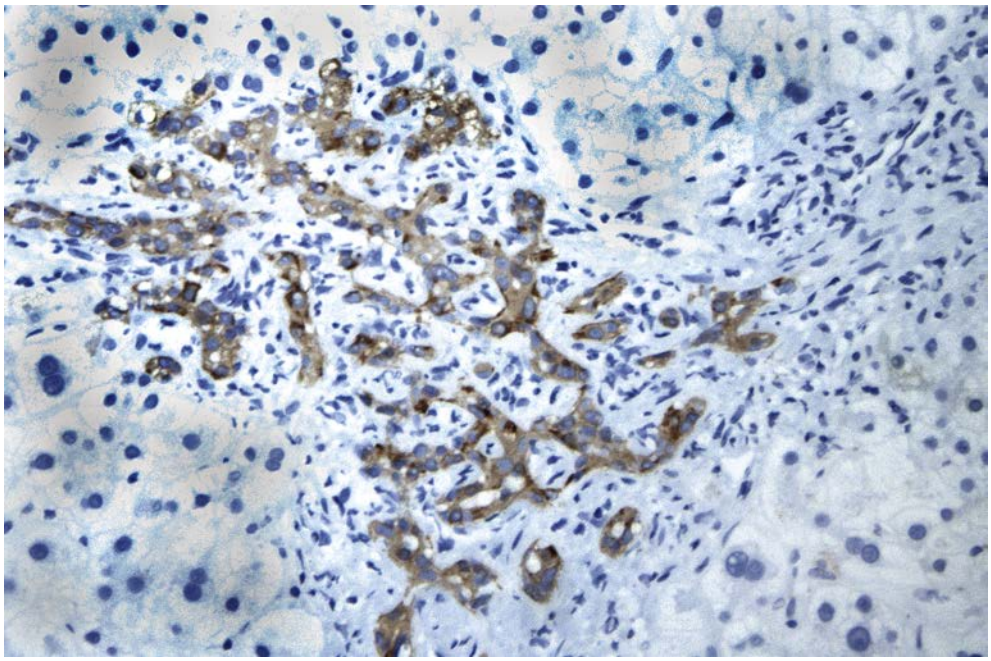

Fig. 5.26 Stage 3 primary biliary cholangitis. The ductular reaction is highlighted by cytokeratin 7 immunostaining of a serial section of the same biopsy shown in **Fig. 5.24**. (Needle biopsy, specific immunoperoxidase.)

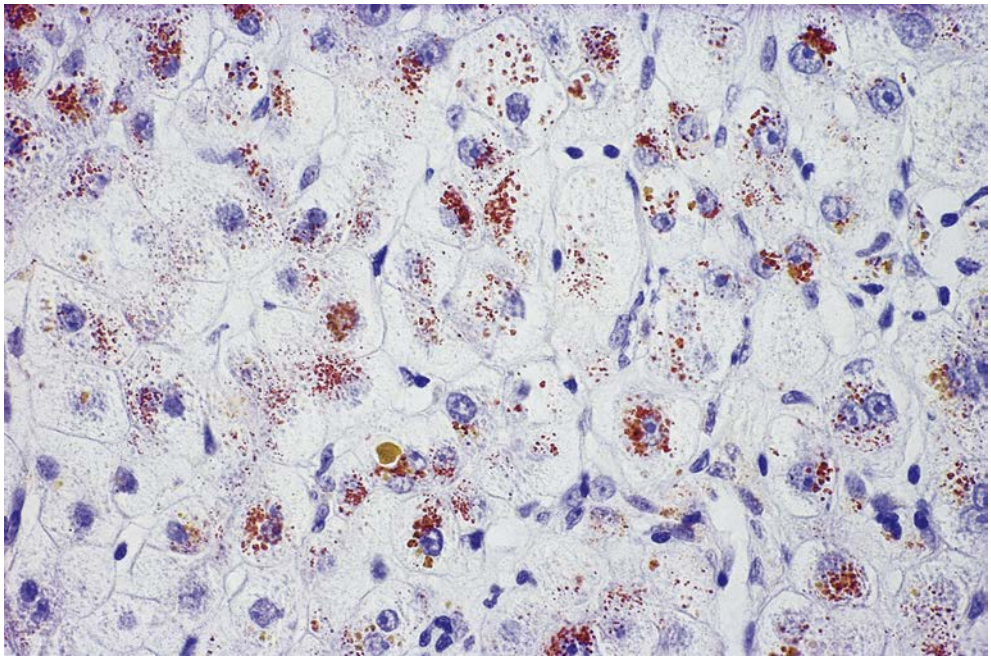

Fig. 5.27 Primary biliary cholangitis. Heavy accumulation of copper-rich granules is seen in hepatocytes. Note the different colour of the canalicular bile thrombus slightly below centre. (Needle biopsy, rhodanine.)

Loss of bile ducts also leads to the chronic form of cholestasis marked by swelling of hepatocytes, bile staining, Mallory body formation and accumulation of copper (**Fig. 5.27**) and copper-associated protein (**Fig. 5.28**). Bile plugs are sometimes seen in canaliculi in the affected areas around portal tracts and septa, but more widespread canalicular cholestasis

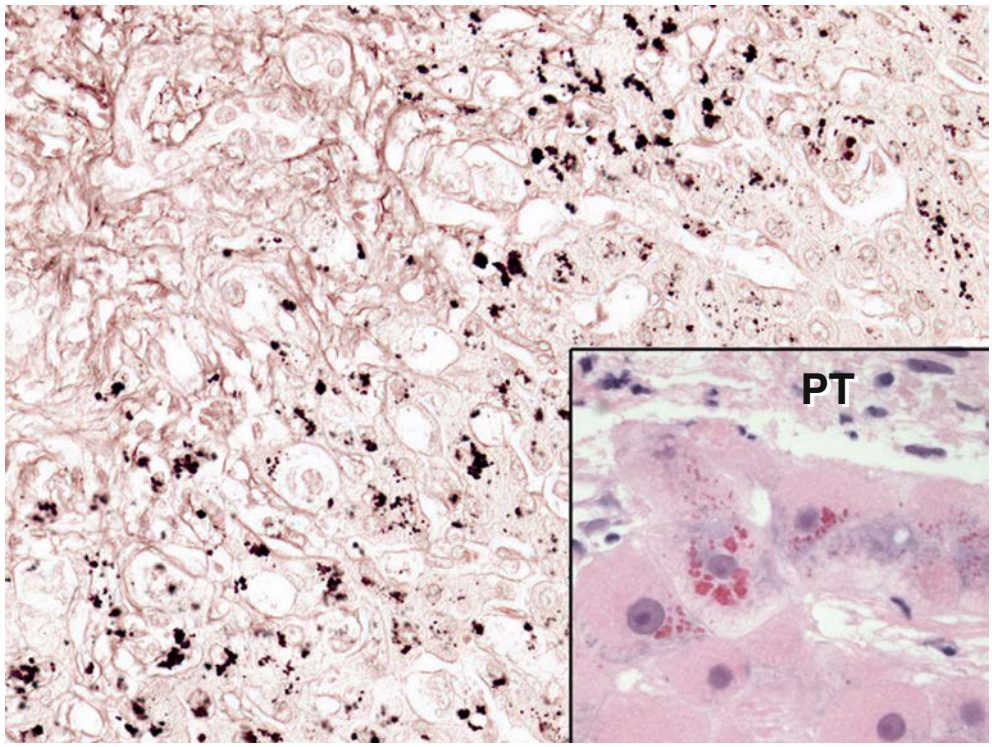

Fig. 5.28 Primary biliary cholangitis. Granular deposits of copper-associated protein have accumulated in hepatocytes near a fibrous septum (below) at a late stage of the disease. (Needle biopsy, Orcein.) Inset: Copper-associated protein in hepatocytes near the portal tract (PT) appears as fine red cytoplasmic granules. (Needle biopsy, H&E.)

often reflects hepatocellular failure or associated sepsis. There may be many lipid-laden macrophages, forming diffuse or localised xanthomas.

In addition to the cholestatic features described earlier, interface hepatitis of the classical, lymphoplasmacytic type is common in the progressive stage of PBC.[21] The infiltrate is rich in activated T cells.[131,133,134] Because this hepatocellular component is a regular feature of PBC, the finding of interface hepatitis together with the biliary features of PBC should not by itself lead to the diagnosis of an overlap syndrome (discussed later). Lymphocytes also form bridge-like extensions into the acini, and may be the forerunners of fibrous septa.[135] An increased number of intrahepatic mast cells is also present, which may have contributed to portal tract fibrosis.[60] Necrosis of perivenular hepatocytes has been noted.[120] There is therefore a histological resemblance to hepatitis. Hepatocellular dysplasia of small- or large-cell type may be present.[124]

The combination of cholestatic and hepatitic processes leads to increasing fibrosis. Portal inflammation diminishes, but lymphoid aggregates continue to mark the former sites of bile ducts (**Fig. 5.29**). Septa extend from the portal tracts and eventually come to link portal tracts to each other and to terminal hepatic venules.[135] In patients in whom the biliary and cholestatic features predominate, the cirrhosis which ultimately develops is generally of the biliary type. When hepatitic features predominate, the cirrhosis tends to be of posthepatitis type. All combinations of the two patterns may be seen.[136] Nodules often develop unevenly throughout the liver, so that nodular areas with the appearance of cirrhosis coexist with areas in which the acinar architecture remains preserved.

The **differential diagnosis of the progressive lesion** includes **PSC** and **other forms of chronic biliary disease** on the one hand and **chronic hepatitis** on the other.[137] The differentiation from primary PSC was discussed earlier; in the later stages of the two diseases it is often impossible to make the distinction histologically. With respect to other forms of chronic biliary obstruction and chronic hepatitis, the most important observation is that bile-duct numbers remain normal in both, whereas they are characteristically reduced in PBC and in PSC

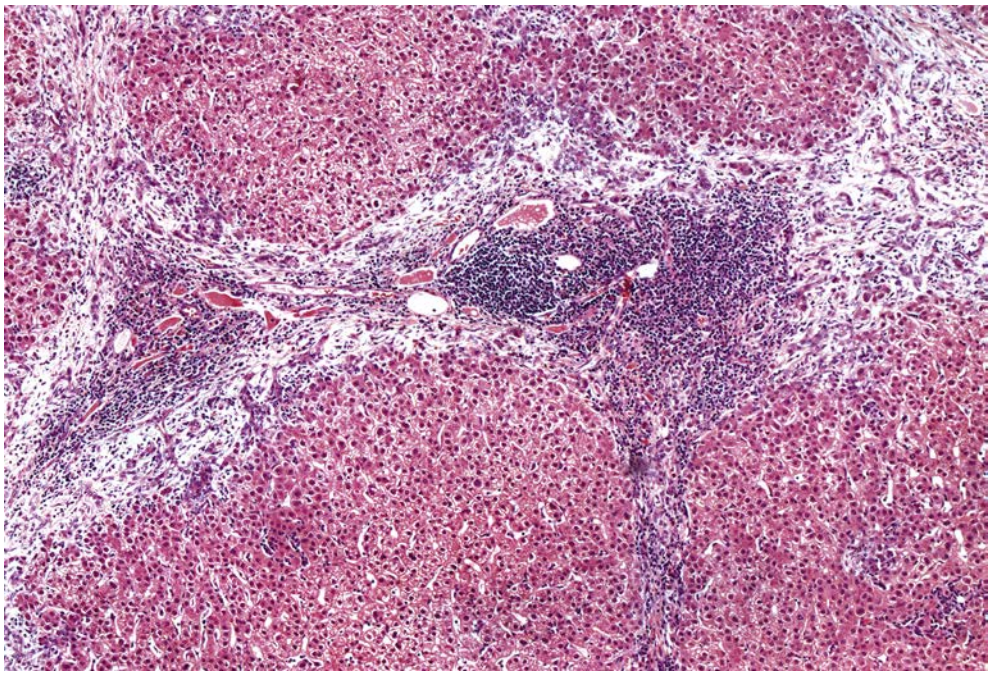

Fig. 5.29 Primary biliary cholangitis. There is extensive scarring without nodule formation. Aggregates of lymphocytes mark the former sites of bile ducts, as in **Fig. 5.22**. (Postmortem liver, H&E.)

(**Table 5.2**). Granulomas favour PBC over chronic hepatitis, as does chronic cholestasis, particularly when it is seen in the absence of cirrhosis.[74] There are also rare cases of concomitant PBC and sarcoidosis with granulomas which may present the pathologist with unique diagnostic interpretive problems.[138] Difficulties remain even after these many factors are taken into account, especially if the biopsy specimen is small or fragmented. They can usually be resolved by consideration of the clinical context and laboratory investigations; a middle-aged woman with itching, high levels of serum alkaline phosphatase and AMAs is unlikely to be suffering from chronic viral hepatitis. There are, however, unusual cases which present as overlap syndromes, discussed briefly in the following section.

Table 5.2 Causes of bile-duct damage and loss

Loss of ducts	Little or no loss
Primary sclerosing cholangitis	Bile-duct obstruction
Primary biliary cirrhosis	Viral hepatitis
Idiopathic ductopenia	Drug jaundice
Graft-versus-host disease	Parasitic duct disease
Chronic rejection of liver grafts	
Sarcoidosis	
Drug jaundice*	

*Note that, while many drugs produce bile-duct injury without loss, there are also well-described cases where ductopenia and chronic cholestatic disease (sometimes requiring liver transplantation) are sequelae of drug hepatotoxicity.

Overlap syndromes, transitional diseases and autoimmune cholangitis

Establishing a clear-cut diagnosis of PBC or PSC is occasionally problematic when an unusually severe degree of lymphoplasmacytic interface hepatitis is superimposed on otherwise typical histopathological features of either disease (**Fig. 5.30**), raising the possibility of an **overlap syndrome**[139,140] with AIH. Such patients may show a mix of serum autoantibodies (some of which may be merely non-specific markers of immune disease), further clouding the diagnosis. In some instances there appears to be a genetic predilection for the hepatitic component, as in certain cases of PBC where a specific histocompatibility profile is present.[141] Rendering a diagnosis of either PBC/AIH or PSC/AIH overlap syndrome therefore requires close consultation between pathologist and clinician, taking into account and

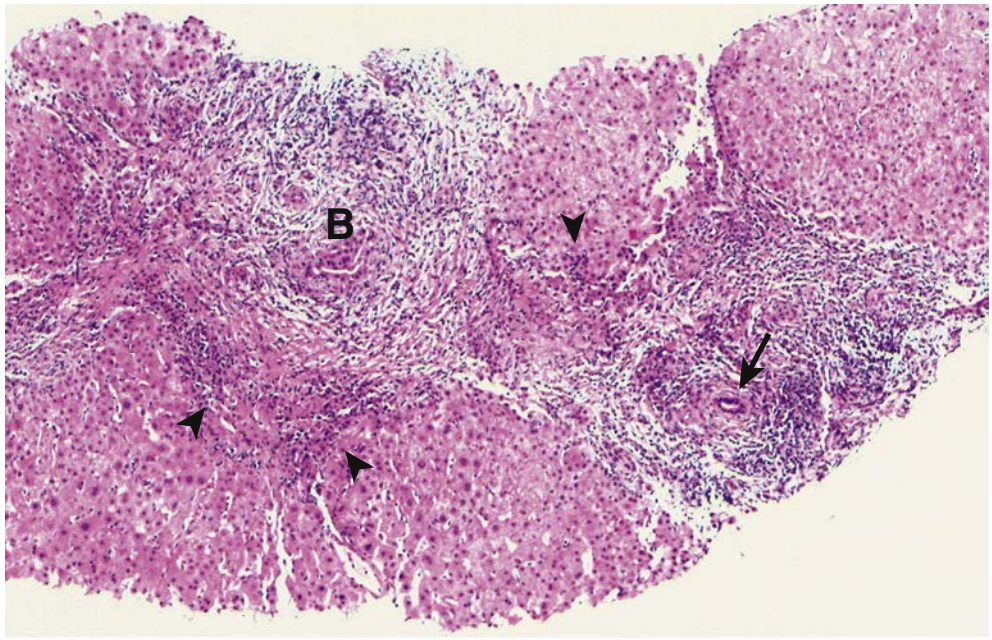

Fig. 5.30 Autoimmune hepatitis-primary sclerosing cholangitis overlap syndrome. Biopsy from a young patient with ulcerative colitis and biliary tree abnormalities on cholangiogram, but clinicopathological and serological features suggested an overlap with autoimmune hepatitis. The biopsy features of biliary disease include abnormal bile-duct morphology (B), portal oedema and periduct fibrosis (arrow), while the extensive interface hepatitis (arrowheads) suggests an autoimmune component. (Needle biopsy, H&E.)

appropriately weighting the biopsy features, serological and biochemical data, and cholangiographic findings.[63,139] The International Autoimmune Hepatitis Group recommends categorizing putative 'overlap' cases according to the predominating features such as AIH, PBC or PSC/small-duct PSC.[142] Use of the clinicopathological scoring systems developed for AIH may be helpful in some cases,[143–145] although not devised for this purpose.[142] The diagnostic dilemma of putative 'overlap' cases resides in the current imprecision in defining the individual outermost diagnostic borders of PBC, PSC and AIH,[146] insensitivity of tests used to detect serum mitochondrial antibodies,[95] non-specific generation of various autoantibodies and superimposition of histological features that may cloud the true diagnosis. A pathological diagnosis of overlap syndrome should therefore be made with due restraint whenever possible and only after careful examination of all the biopsy features, including bile-duct morphology, evidence of chronic cholestasis, lobular necroinflammatory changes and interface hepatitis. Paediatric liver biopsies from children with liver disease, serum autoantibodies and a clinical diagnosis suggestive of AIH require particularly careful microscopic evaluation for exclusion of overlap of AIH and PSC (**ASC**), an immune disorder which is significantly more common in children than adults.[147,148] Liver biopsy in ASC may demonstrate only the changes of chronic hepatitis without biliary features in a significant percentage of children with demonstrated biliary lesions on cholangiography.[148]

The interrelatedness of PBC, PSC and AIH as disorders of cellular immunity[149] is further highlighted by descriptions of clinical **transition** from one form to another. Such examples include cases of PBC progressing to AIH,[150,151] AIH progressing to PSC,[152] paediatric PSC patients with autoimmune serological and histopathological features[153] and transplanted PBC patients who develop AIH in their allografts.[154]

A number of patients with typical biopsy features of PBC but no demonstrable serum AMAs have been described as cases of **autoimmune cholangitis** because of coexistent

antinuclear or other autoantibodies, occasional corticosteroid responsiveness and other features that suggest an autoimmune clinical profile.[155] Such cases may reflect problems in the sensitivity of current mitochondrial antibody tests and are now usually considered to be examples of AMA-negative PBC.[95,139]

Other disorders with intrahepatic bile-duct loss

As indicated earlier, the loss of significant numbers of intrahepatic bile ducts (ductopenia) can be seen not only in PBC and PSC but also in several conditions listed in **Box 5.1**. In addition to these, there are patients in whom the pathogenesis of duct loss is poorly understood. Some of these may represent later stages of childhood **non-syndromatic paucity of intrahepatic bile ducts.**[156] In patients with **idiopathic adulthood ductopenia**—predominantly males with cholestatic biochemical profiles—duct loss could also be the end result of small-duct PSC or due to bile-duct damage associated with chronic hepatitis C[157] or autoimmune cholangitis.[158] Rarely, idiopathic adulthood ductopenia is familial.[159] Ductopenia due to the paraneoplastic effects of **Hodgkin's disease** was reported.[160] A group of asymptomatic patients with idiopathic ductopenia and elevated serum γ-glutamyl transferase activity has also been described.[161]

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CHAPTER 6

Acute Viral Hepatitis

Introduction

Acute hepatitis is not usually an indication for liver biopsy. There are, however, at least three reasons why pathologists sometimes receive liver biopsy samples from patients with acute hepatitis. First, there may be doubt about the clinical diagnosis, or even a mistaken working diagnosis. Second, a diagnosis of hepatitis may be well established but the clinician needs information on the stage of the disease or its severity. Third, the patient may have received a liver transplant and the pathologist is being asked to help decide if symptoms or biochemical abnormalities are due to recurrent (or new) viral hepatitis or to some other cause such as rejection. For all these reasons, a knowledge of the pathology of acute hepatitis is essential. There is a further reason, no less important than the others: without a knowledge of acute hepatitis, the pathologist cannot hope to understand chronic hepatitis and cirrhosis, together the cause of most liver disease in the world. This chapter describes acute viral hepatitis and its immediate sequelae in the immunocompetent patient. The specific problems of diagnosing hepatitis in an immunosuppressed patient after transplantation are reviewed in **Chapter 16**.

The hepatitis viruses are listed in **Table 6.1**. While several other candidates have been extensively investigated in recent years, none has so far been established as a definite cause of viral hepatitis, and most episodes of acute and chronic hepatitis can be attributed to one of the viruses listed, to autoimmune hepatitis (**Ch. 9**) or to a hepatotoxic agent (**Ch. 8**). An exception to this statement is fulminant hepatitis, the cause of which cannot currently be established in a substantial minority of patients,[1–3] including children.[4] Occasionally, a virus more often associated with infection of other organs, such as one of the herpesviruses[5–7] or an adenovirus,[8,9] gives rise to a severe hepatitis. These agents are further discussed in **Chapter 15**. Mild acute hepatitis has been reported in patients infected with the SARS virus (severe acute respiratory syndrome-associated coronavirus).[10,11]

Occasionally, mild serum liver test abnormalities and mild histological hepatitis (bystander hepatitis) with apoptotic bodies, focal necrosis and lymphocytic inflammation are seen in systemic, non-hepatic viral infections such as pulmonary influenza and result from migration to the liver of, and collateral damage by, CD8 T lymphocytes.[12,13]

Pathological features

The essential components of the acute phase of hepatitis are inflammatory-cell infiltration and hepatocellular damage. Other features include cholestasis, Kupffer-cell activation, endotheliitis, bile-duct damage, the ductular reaction and hepatocellular regeneration.

Table 6.1 The hepatitis viruses

Virus	Type	Spread and disease
Hepatitis A (HAV)	RNA hepatovirus	Faecal–oral, acute
Hepatitis B (HBV)	DNA hepadnavirus	Parenteral, acute or chronic
Hepatitis C (HCV)	RNA hepacivirus	Parenteral or sporadic; acute, more often chronic
Hepatitis D (HDV)	RNA deltavirus, defective	Pathogenic when combined with HBV
Hepatitis E (HEV)	RNA virus	Faecal–oral, epidemic or sporadic acute disease

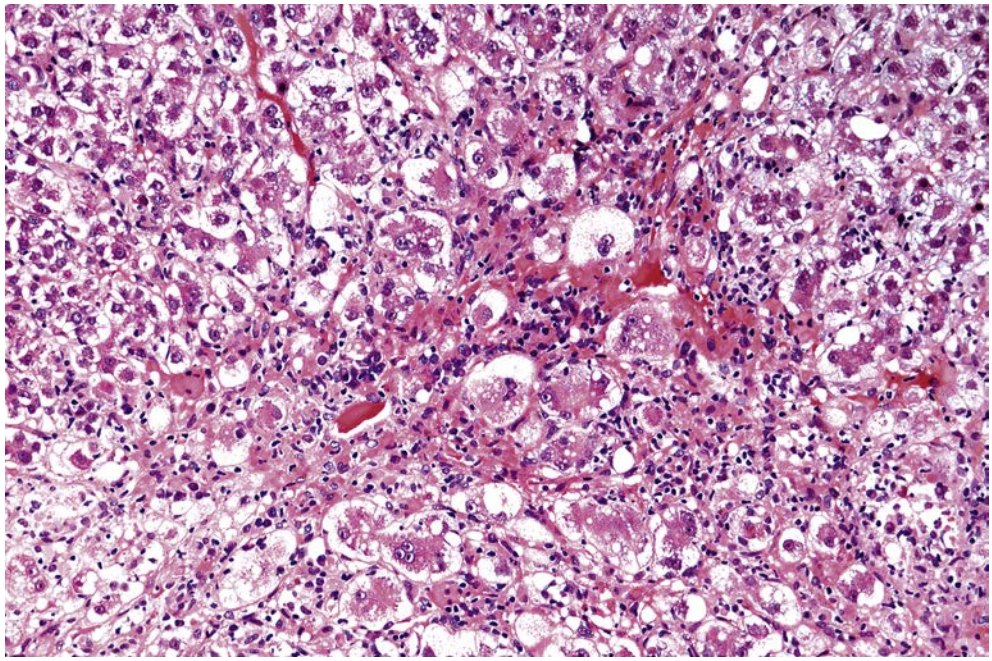

Fig. 6.1 Acute viral hepatitis. Surviving hepatocytes in the perivenular area in the centre of the field are swollen and the area is infiltrated by inflammatory cells. (Needle biopsy, H&E.)

Hepatocellular damage

Changes seen under the light microscope range from minor degrees of cell swelling to cell death. They are accompanied by the inflammatory infiltration described below, reflecting the important role of cellular immunity in the pathogenesis of most forms of hepatitis. Both hepatocellular damage and inflammation are usually most severe in perivenular areas, giving rise to a characteristic histological pattern (**Fig. 6.1**). A periportal pattern of necrosis and inflammation, sometimes seen in hepatitis A, is less common.

The mildest change is cell swelling, and this is probably reversible. The cytoplasm of affected cells is rarified, granular and sometimes finely vacuolated. The more severe degrees of cell swelling are called ballooning degeneration (**Fig. 6.2**). This differs from the feathery degeneration of cholestasis, in which the cytoplasm has a reticular pattern (**see Fig. 5.3**), and from the ballooning in steatohepatitis where the cytoplasm is less granular and more oedematous and 'clarified' (**see Fig. 7.10C**). Other hepatocytes undergo apoptosis, which is an important method of cell death in hepatitis.[14] Shrinkage and increased staining of the cytoplasm, sometimes called acidophilic

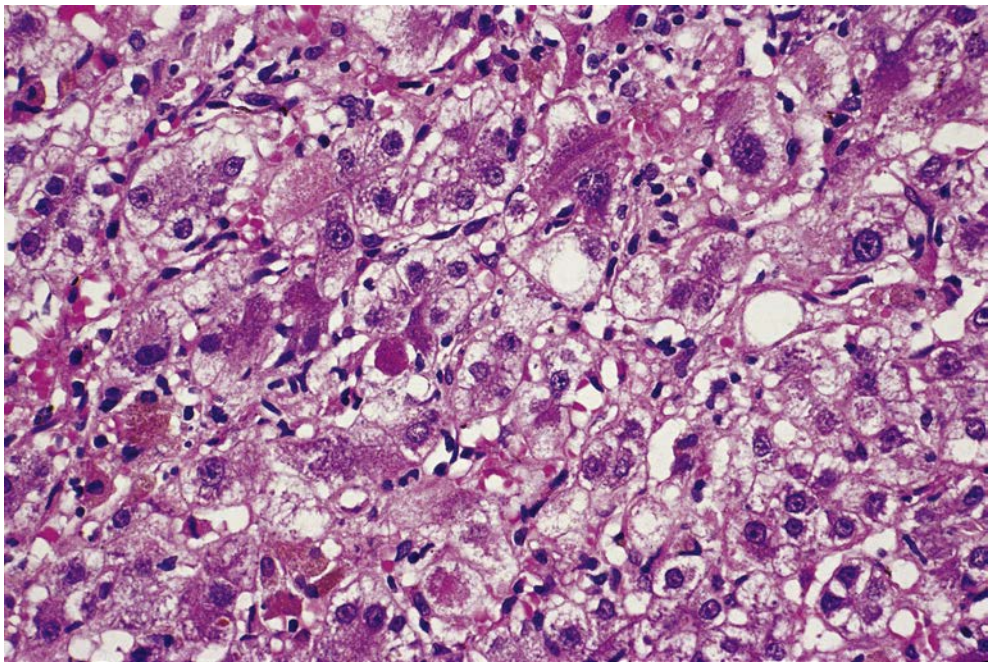

Fig. 6.2 Acute viral hepatitis. Normal liver-cell plate structure is disrupted. Hepatocytes vary in size, and some are ballooned and vacuolated. An apoptotic hepatocyte is seen left of centre. (Needle biopsy, H&E.)

change or degeneration, is probably a precursor of apoptosis, in which the hepatocytes shrink further, become very dense and undergo fragmentation. The apoptotic bodies seen lying free in the sinusoids represent the largest fragments or entire unfragmented apoptotic cells (**Fig. 6.2**). They are also called acidophil bodies or Councilman bodies, Councilman having first described them in yellow fever[15,16] (**Fig. 6.3**). Apoptotic bodies sometimes contain pyknotic nuclear remnants and often appear to bulge beyond the plane of the section. Another form of hepatocellular damage in acute hepatitis is focal (spotty) necrosis, in which liver-cell plates are disrupted or replaced by small groups of lymphocytes and macrophages. Whether these mark a site of necrosis or of apoptosis is not clear; the damage to hepatocytes is deduced from their absence rather than seen. Whatever its mechanism, loss of hepatocytes or liver-cell drop-out, coupled with focal regeneration, leads to a characteristic irregularity of the liver-cell plates, which usually allows acute hepatitis to be distinguished from hepatocellular damage secondary to cholestasis. The loss of hepatocytes also leads to condensation of the extracellular matrix, best seen in reticulin preparations (**Fig. 6.4**).

Hepatocyte nuclei show prominent nucleoli and increased variation in size and may be multiple. When syncytial giant hepatocytes are very prominent, the term giant-cell hepatitis is appropriate.[17,18] This is only rarely of proven viral origin and is also more characteristic of acute hepatitis in neonates. In adults, autoimmune hepatitis and hepatitis C virus with or without human immunodeficiency virus co-infection are important associations.[19–23]

Cholestasis in the form of bile thrombi in canaliculi is common in acute hepatitis but rare in chronic hepatitis, which is diagnostically helpful. It is a result of damage to the bile-secretory apparatus of the hepatocytes, but may also result from interference with bile flow at the level of the portal tracts.[24] The term cholestatic hepatitis is best kept as a clinical description of patients with a prolonged cholestatic course. Mild hepatocellular siderosis or steatosis is occasionally seen.

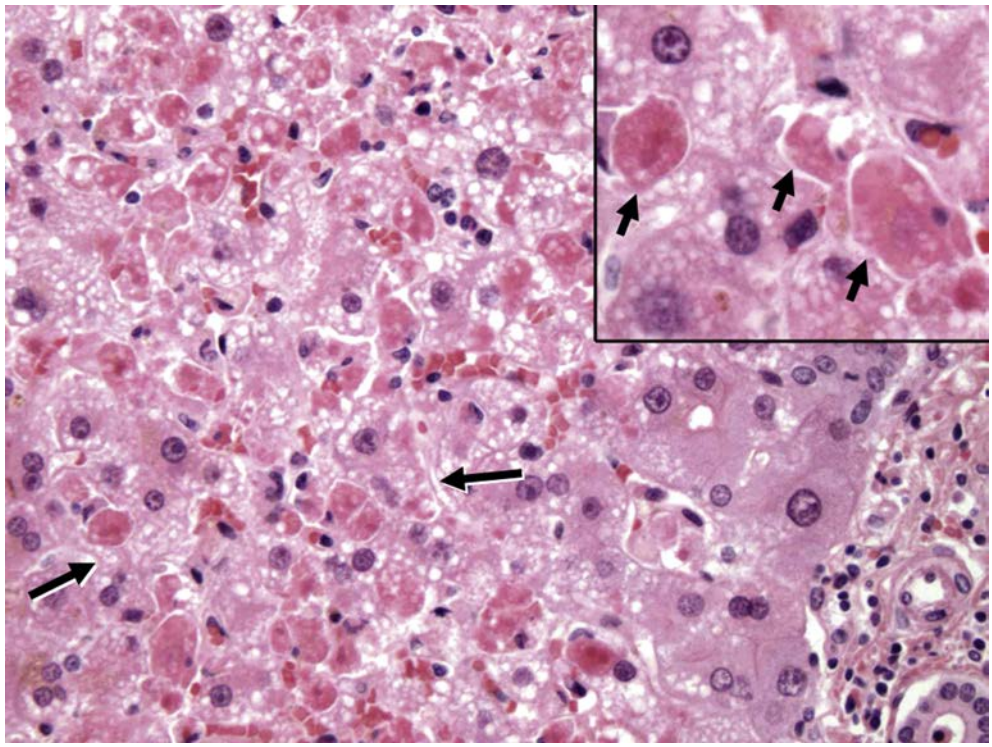

Fig. 6.3 Acute yellow-fever hepatitis. There is prominent mid-zonal necrosis (between arrows) with many apoptotic hepatocytes and scattered lymphocytes. The portal tract at lower right is mildly inflamed and there is relative preservation of periportal parenchyma. Inset: The numerous apoptotic (Councilman) bodies present (arrows) are characteristic of liver involvement in yellow fever. (Case kindly provided by Dr Matthias Szabolcs, New York, NY.)

The inflammatory infiltrate

Unlike classic acute inflammation, viral hepatitis is characterised by a mainly lymphocytic infiltrate within the parenchyma and portal tracts. In acute hepatitis, the most conspicuous inflammation is usually perivenular. The extent of portal inflammation is very variable, and portal tracts may be either normal in size or expanded. The larger conducting tracts are often spared. The edges of small portal tracts may be well defined or blurred by outward extension of the infiltrate. This so-called spillover resembles the interface hepatitis of chronic hepatitis (Ch. 9) and may be difficult to distinguish from it. The parenchymal changes, clinical history and virological findings usually make the correct diagnosis clear.

While most of the infiltrating cells in acute hepatitis are small T lymphocytes,[25] plasma cells may also be prominent,[26] and there are often a few neutrophils and eosinophils. The plasma cells do not necessarily indicate autoimmune hepatitis, nor do a few eosinophils prove a diagnosis of drug injury. Kupffer cells and other macrophages accumulate and enlarge, many of them forming discrete clumps together with lymphocytes. They may contain tan-brown ceroid pigment, staining with periodic acid–Schiff (PAS) agent after diastase digestion (Fig. 6.5). They may also contain stainable iron (Fig. 6.6), but this is less common.

Sinusoidal and venular endothelial cells also take part in the hepatitic process. Sinusoidal endothelial cells become swollen and may contain dense iron-positive

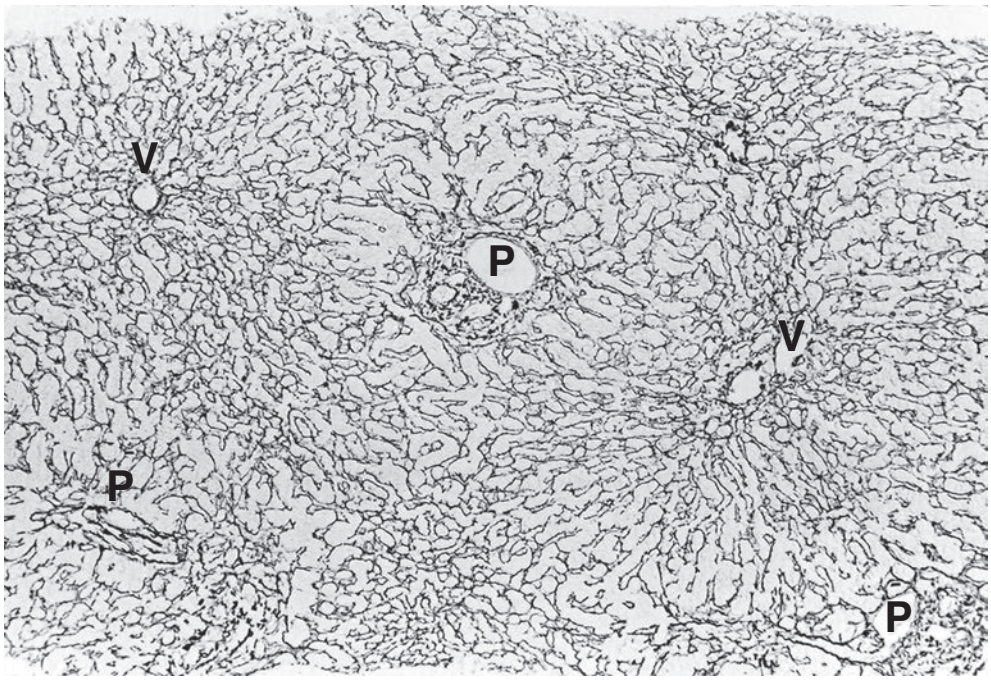

Fig. 6.4 Acute viral hepatitis. The reticulin framework is condensed near the efferent venules (V) but not immediately around the portal tracts (P). (Needle biopsy, reticulin.)

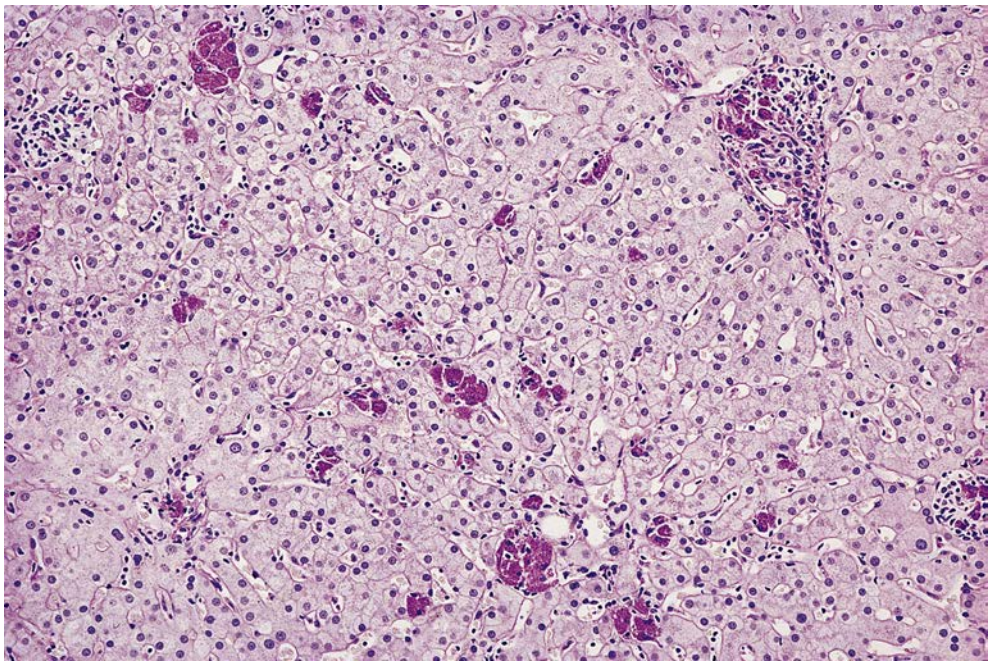

Fig. 6.5 Acute viral hepatitis. Macrophages contain diastase periodic acid–Schiff (PAS)-positive material. (Needle biopsy, diastase–PAS.)

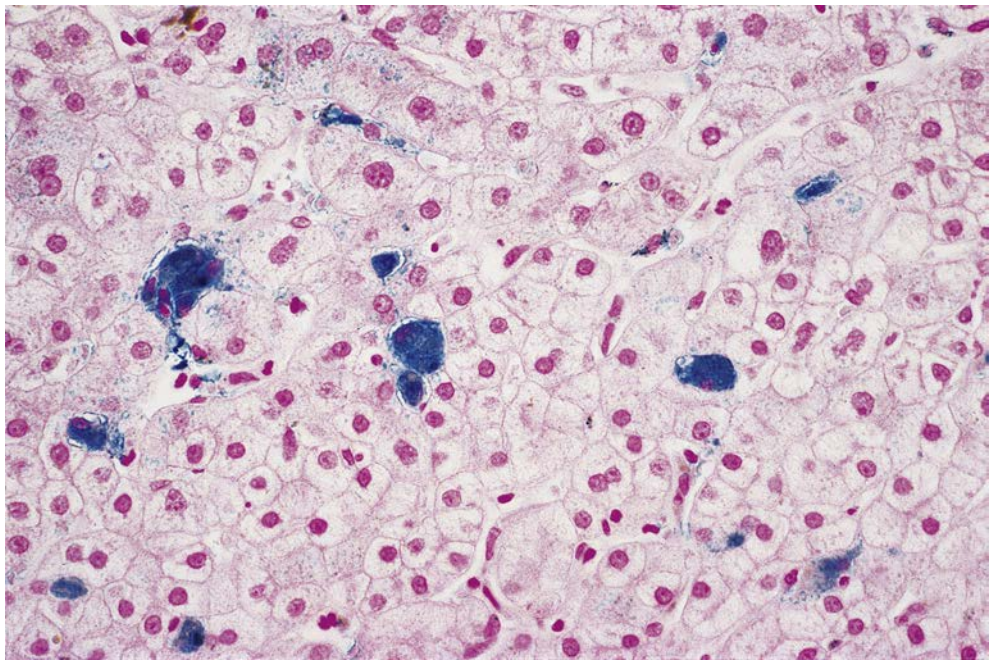

Fig. 6.6 Acute viral hepatitis. Enlarged macrophages are strongly iron positive. Some endothelial cells also contain dense Perls' stain-positive granules. (Section kindly provided by Dr Susan Davies, Cambridge, UK.) (Needle biopsy, Perls' stain.)

granules[27] (Fig. 6.6). Terminal hepatic venules may show disruption of the endothelium and lymphocytic infiltration.

Portal changes

In contrast to chronic hepatitis, the parenchymal changes dominate the picture, but there is always some portal inflammation, affecting most or all of the small portal tracts (Fig. 6.7). The density of the infiltrate varies. Interlobular bile ducts may show abnormalities, including irregularity, crowding and stratification of the epithelium, cytoplasmic vacuolation and infiltration by lymphocytes (Fig. 6.8). These changes, together with formation of dense lymphoid structures (aggregates and follicles), are most often seen in hepatitis C. Bile-duct loss (ductopenia) is very rare.

Histological variants

The histological changes in acute hepatitis are infinitely variable, but a few patterns deserve special mention. These are confluent necrosis, bridging necrosis, necrosis of entire lobules and periportal necrosis.

Confluent necrosis signifies death of a substantial area of the parenchyma. Focal as opposed to zonal areas of confluent necrosis haphazardly distributed in relation to lobular zones are more likely to be due to causes other than acute viral hepatitis; possibilities to be considered include opportunistic infections with herpes simplex or zoster viruses and lymphoma. **Bridging necrosis** (Figs 6.9 and 6.10, **and see** Fig. 4.8) is the term given to confluent necrosis linking terminal venules to portal tracts. A possible explanation for this location is that it represents the entire zone 3 of an acinus, a view supported by the curved shape of many bridges. Bridging necrosis is a manifestation of severe acute hepatitis, but its distribution even within a single biopsy may be irregular. Necrosis and inflammation linking adjacent portal tracts without involvement of terminal venules should not strictly be

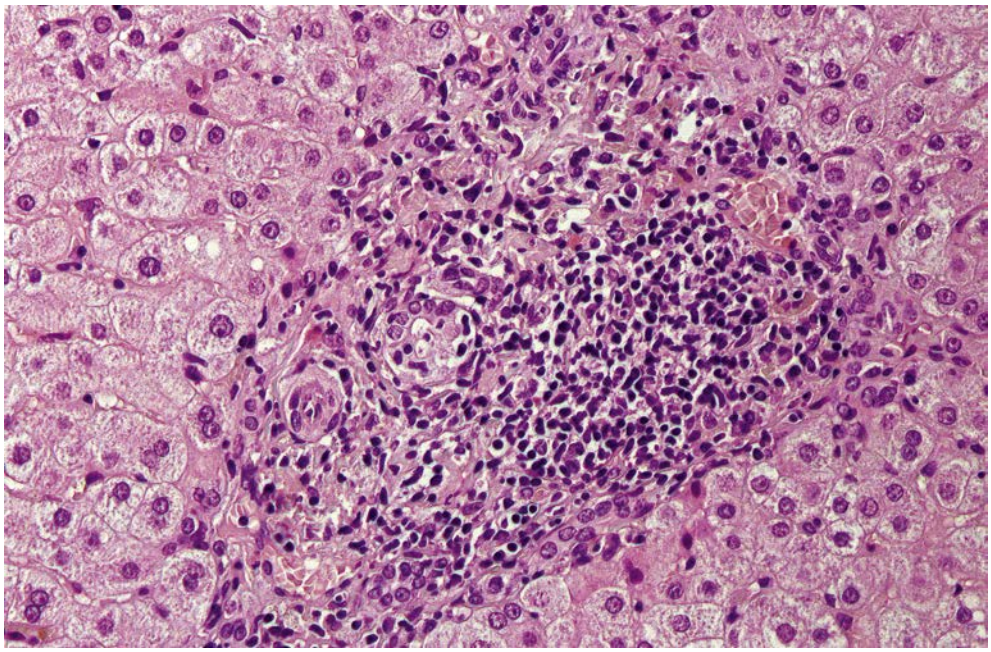

Fig. 6.7 Acute viral hepatitis. A portal tract is infiltrated by inflammatory cells, mainly lymphocytes. In places the infiltrate extends a short way into the adjacent parenchyma. (Needle biopsy, H&E.)

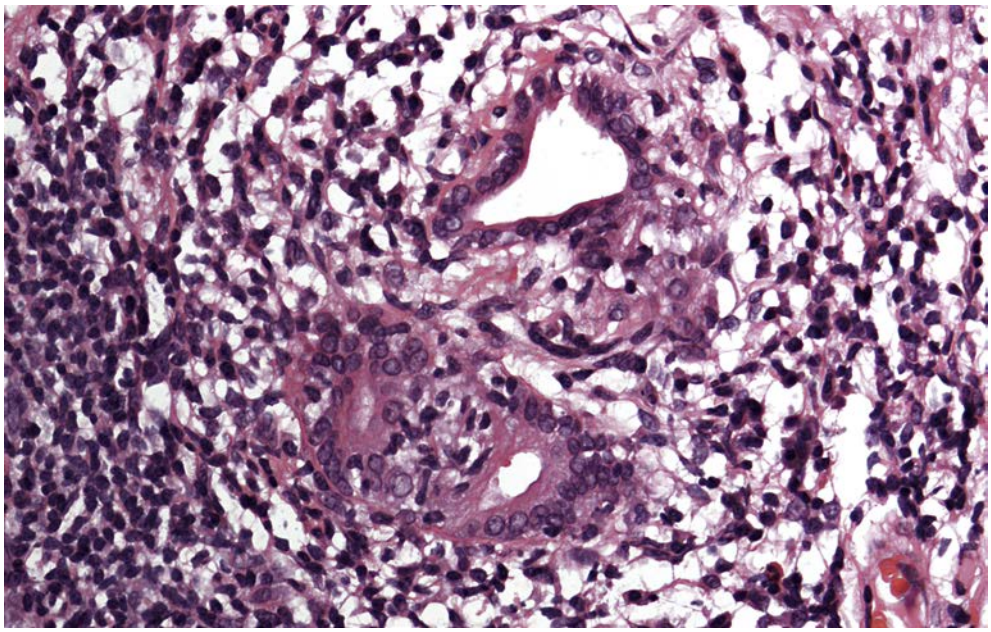

Fig. 6.8 Acute viral hepatitis. Bile-duct epithelium is irregular and infiltrated by lymphocytes. The upper duct profile shows epithelial atrophy and dilatation. (Wedge biopsy, H&E.)

called bridging because it almost certainly has different pathogenetic significance; it results from widening of portal tracts, with or without periportal necrosis.

Bridges of confluent necrosis with subsequent collapse may be mistaken for the septa of chronic liver disease. In making the important distinction between them, the pathologist is often helped by stains for elastic tissue. Unlike stains for collagens, these normally give negative results in the parenchyma, but elastic tissue accumulates as septa age.[28] Recent collapse is therefore negative (**Fig. 6.11**), whereas old septa are positive. Substantial amounts

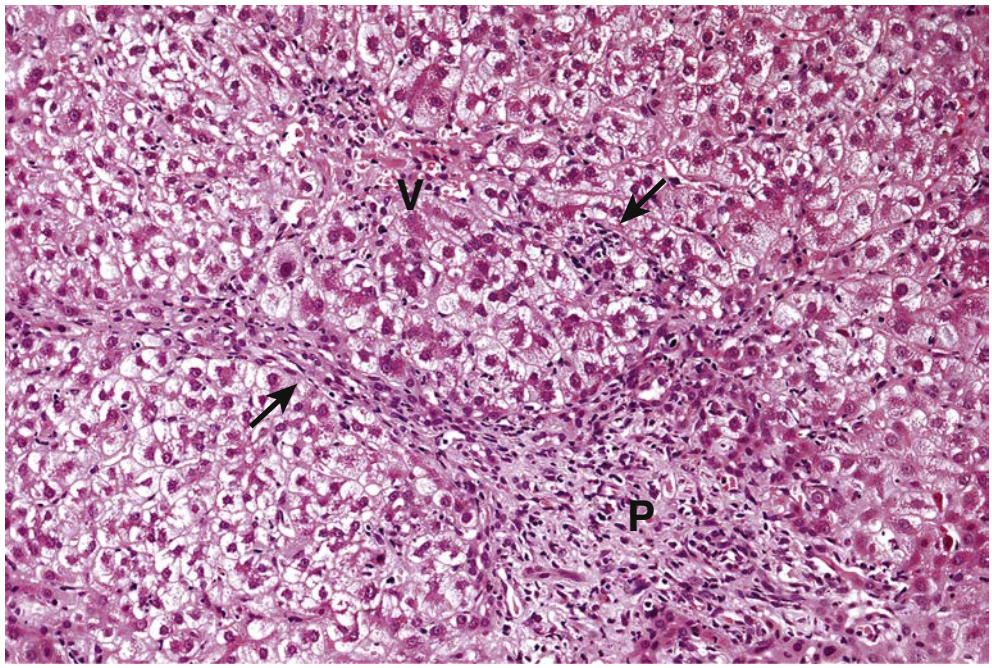

Fig. 6.9 Acute viral hepatitis: bridging necrosis. Two curved lines of collapse (arrows) extend from a portal tract (P). An efferent venule (V) is seen top centre. (Needle biopsy, H&E.)

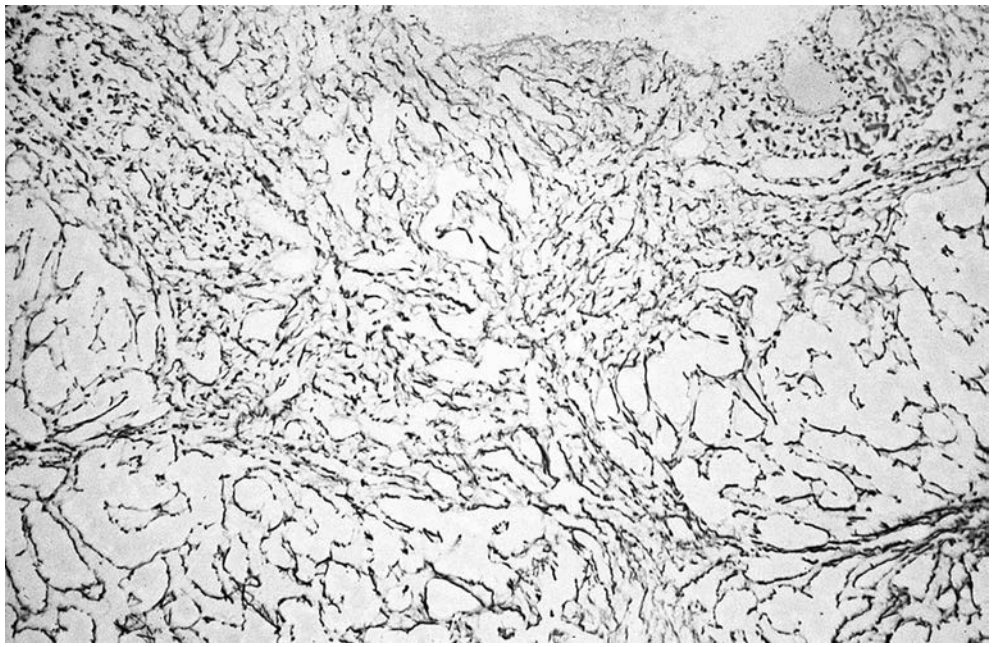

Fig. 6.10 Acute viral hepatitis: bridging necrosis. Recent collapse following confluent necrosis is seen as condensation of reticulin, mimicking fibrosis. (Needle biopsy, reticulin.)

of elastic tissue take months or years to accumulate, but small amounts can be detected by sensitive methods such as Victoria blue as early as 1 or 2 months after onset of hepatitis.[29]

In a minority of patients with acute viral hepatitis, confluent necrosis extends throughout entire lobules or acini (**panlobular** or **panacinar necrosis**) or several adjacent ones (**multilobular** or **multiacinar necrosis**). This is a common feature in patients with

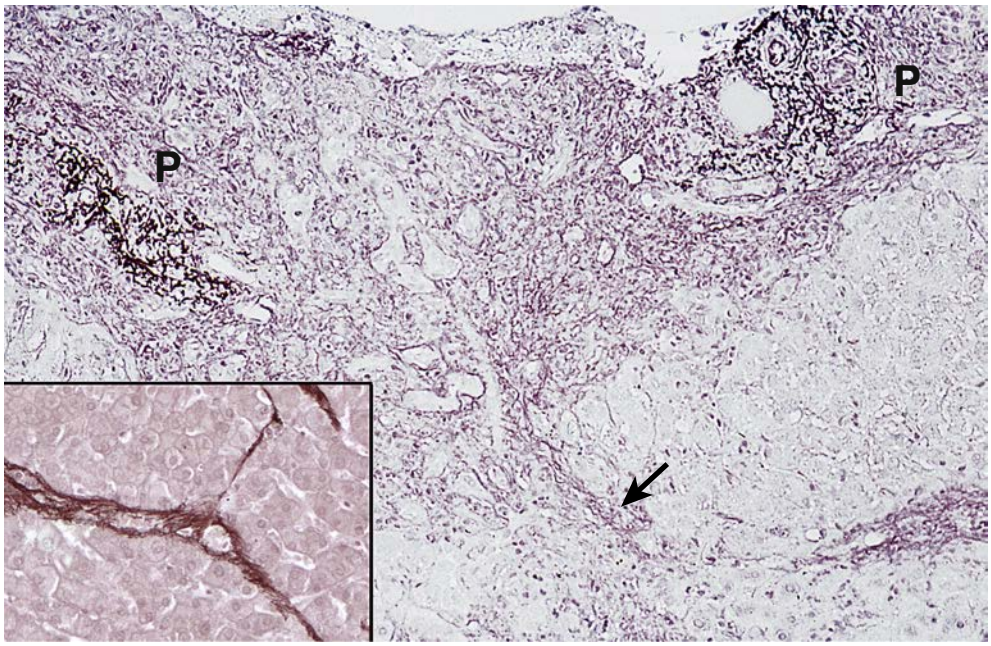

Fig. 6.11 Acute hepatitis: bridging necrosis. The field is the same as that shown in **Fig. 6.10**. A stain for elastic fibres is positive in two portal tracts (P) but not in the intervening area of collapse. A necrotic bridge (arrow) is also negative. Inset: This contrasts with an elastic fibre-rich septum in chronic liver disease. (Needle biopsy, orcein.)

fulminant hepatitis. The term 'massive necrosis' is also sometimes used, but can be misleading in so far as a needle biopsy specimen may not be representative of the liver as a whole and can lead to over- or underestimation of the true extent of liver damage.[30] This throws doubt on the usefulness of liver biopsy as a means of assessing prognosis in severe acute hepatitis. Sometimes multilobular necrosis involves only the subcapsular zone, and a small needle specimen may then give a falsely pessimistic picture (**see Fig. 1.3**). In multilobular necrosis the parenchyma is replaced by collapsed stroma, inflammatory cells and activated macrophages (**Fig. 6.12**). Around the surviving portal tracts there are prominent duct-like structures, some of which probably represent proliferation of pluripotential progenitor cells[31–33] (**see Fig. 4.13D**). 'Late-onset hepatic failure' is a term used for patients developing encephalopathy between 8 and 24 weeks after onset of symptoms.[34] Study of liver biopsies and explanted livers from these patients has shown a consistent pattern of map-like necrosis together with areas of nodular regeneration.

Periportal necrosis rather than the more usual perivenular necrosis is a feature in some patients with hepatitis A (discussed later).

Individual causes of viral hepatitis

There are more similarities than differences between hepatitis types A, B, C, D and E, but certain patterns are more common in one type than another and are described here. They do not allow the pathologist to identify the cause of the hepatitis on histological appearance alone. The picture may be confused by the presence of more than one virus, or by additional damage resulting from alcohol abuse.

Hepatitis A

Two main patterns are described, occurring separately or together.[35–37] One is a histological picture of perivenular cholestasis with little liver-cell damage or inflammation, easily

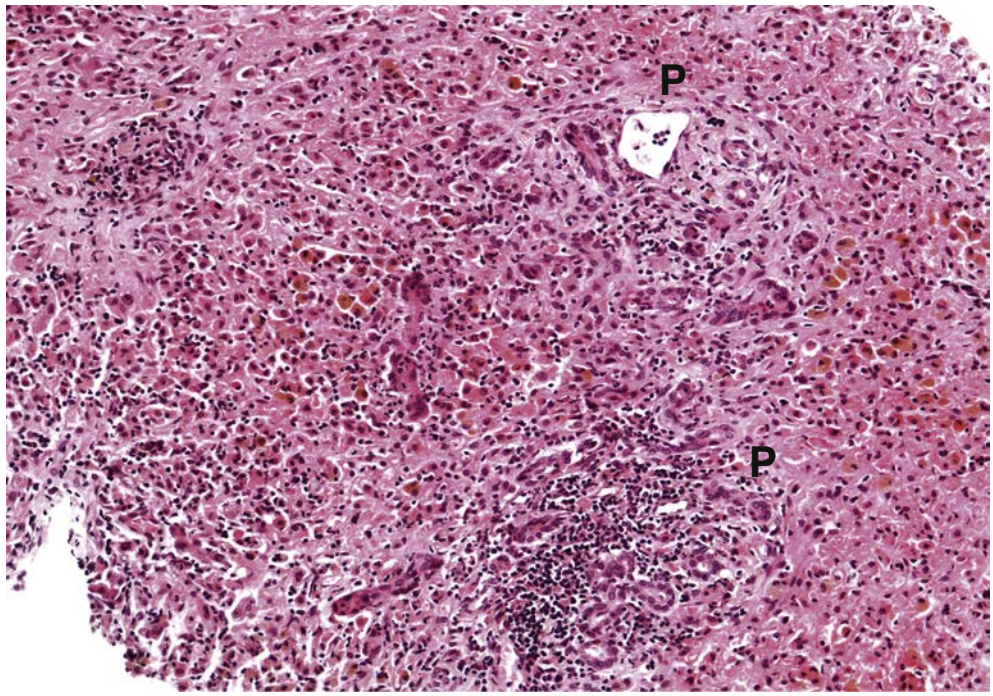

Fig. 6.12 Acute viral hepatitis: multilobular necrosis. Portal tracts (P) can be identified, but the parenchyma has been replaced by inflammatory cells, necrotic debris and duct-like structures. (Needle biopsy, H&E.)

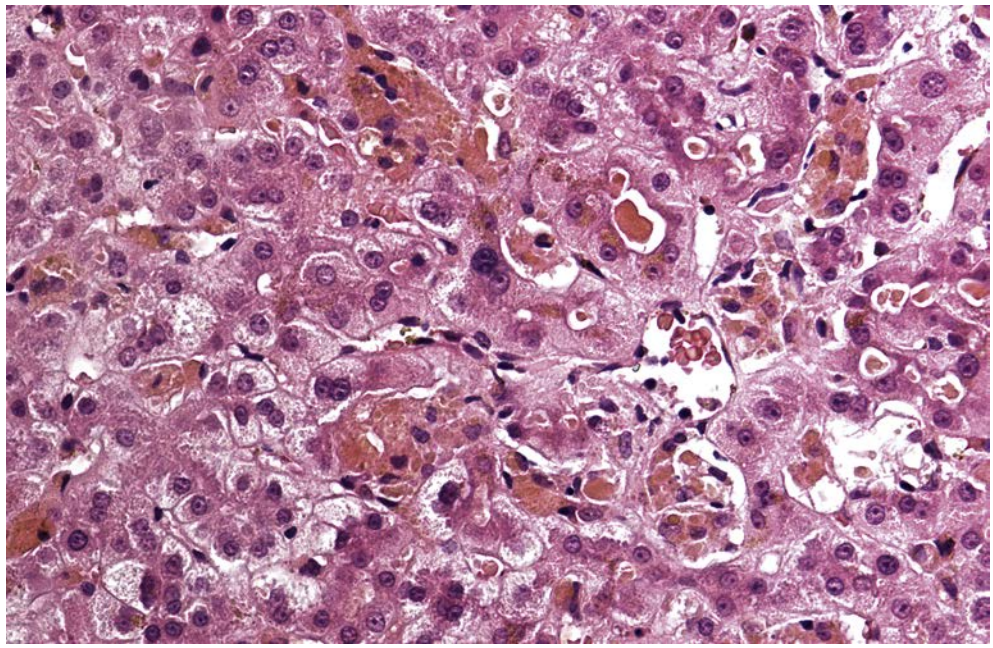

Fig. 6.13 Hepatitis A. Perivenular area showing irregularity of liver-cell plates and cholestasis but only mild inflammatory infiltration. (Needle biopsy, H&E.)

mistaken for other causes of cholestasis (Fig. 6.13). The second is a hepatitis with periportal necrosis and a dense portal infiltrate which includes abundant, often aggregated plasma cells (Fig. 6.14). These two patterns may be related, the cholestasis resulting from interruption of bile flow by the periportal necrosis.[23] Other patterns of hepatitis as described earlier are also found, but fulminant hepatitis with multilobular necrosis is rare. Extensive

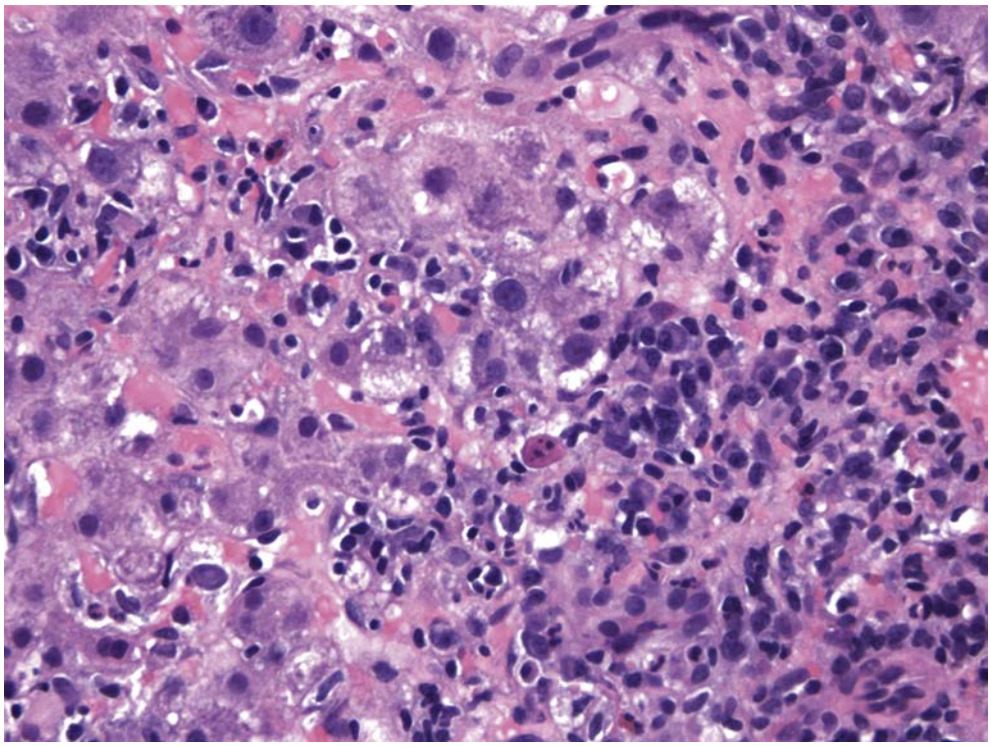

Fig. 6.14 Hepatitis A. The portal area at right is heavily infiltrated by lymphocytes and plasma cells, some of which extend into the adjacent parenchyma. The limiting plate is irregular. The picture resembles that of chronic hepatitis with interface hepatitis. (Needle biopsy, H&E.)

microvesicular change of hepatocytes, previously described in hepatitis D infection, has been seen also in severe acute hepatitis A (**Fig. 6.15**). Fibrin-ring granulomas have been reported.[38,39] A chronic course[40] is very rare.

Hepatitis B

The histological appearances are broadly similar to those of other forms of viral hepatitis. Some of the differences reported in the literature may well reflect patient selection rather than features specific for hepatitis B virus (HBV) infection. However, lymphocytes and macrophages sometimes lie in close contact with hepatocytes (peripolesis) or even invaginate them deeply (emperipolesis), which probably reflects the immunological nature of the cell damage. In a comparative study, periportal inflammation tended to be more severe in acute hepatitis B than in hepatitis C.[41] Liver cells and their nuclei may show a moderate degree of pleomorphism. In most cases of acute hepatitis, the hepatitis B core and surface antigens (HBcAg and HBsAg) are either not demonstrable or very sparse, but in one study of livers infected with an HBV mutant,[42] HBsAg could be demonstrated by immunostaining in over half of the patients and HBcAg in a minority. The presence of ground-glass hepatocytes (**Ch. 9**) or positive staining of surface material with Victoria blue or orcein indicates chronic disease. Recurrence of HBV infection after liver transplantation is an exception to this rule, both antigens being found in large amounts (**see Ch. 16**). In parenterally transmitted hepatitis, including types B and C, birefringent spicules of talc may be found in portal tracts as a result of intravenous drug abuse.[42]

Following clinical recovery of acute hepatitis B, occult infection and mild histological abnormalities including portal inflammation, focal necrosis, apoptosis and fibrosis may persist for at least a decade.[43]

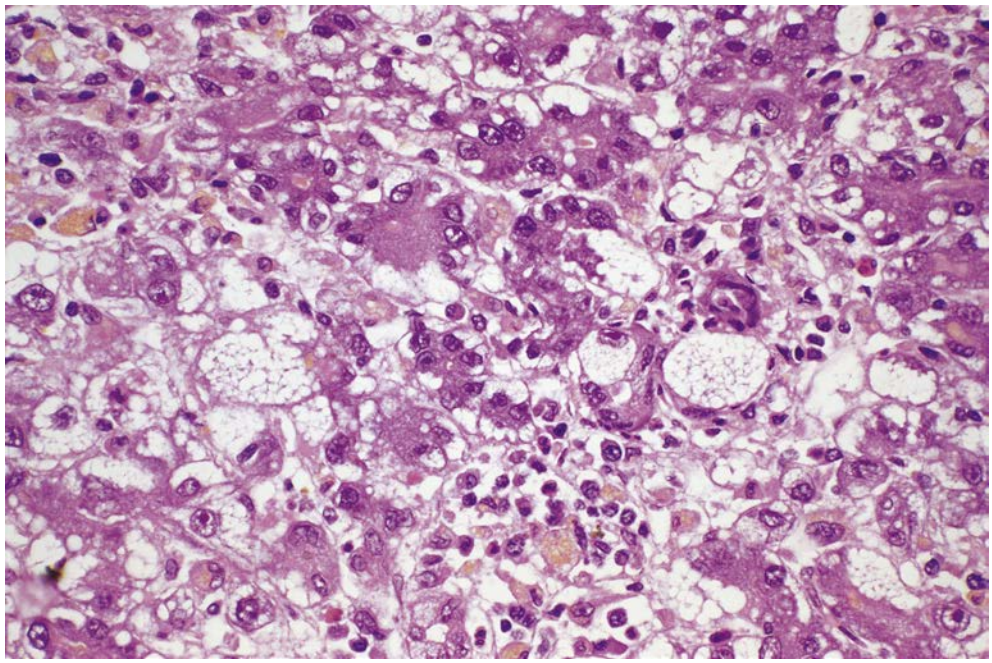

Fig. 6.15 Hepatitis A. In this patient with a clinical picture of fulminant hepatitis, hepatocytes are swollen and microvesicular. There is cholestasis and a lymphocytic infiltrate. (Needle biopsy, H&E.)

Reactivation of a previously occult or quiescent chronic hepatitis B infection may cause changes closely resembling acute hepatitis. In such instances the presence of (1) portal tract lymphoid aggregates, (2) significant lymphoplasmacytic interface hepatitis, (3) any evidence of fibrosis on connective tissue stains and (4) substantial positivity of HBsAg in hepatocytes on immunostaining points to the underlying chronicity of the process.

Hepatitis C

Usually the histological features of hepatitis C are those of any acute hepatitis, but two distinguishing features have been noted. First, there may be prominent infiltration of sinusoids by lymphocytes in the absence of severe liver-cell damage,[44] giving rise to a picture reminiscent of infectious mononucleosis (**Fig. 6.16**). Second, lymphoid follicles and bile-duct damage, features also associated with chronic hepatitis, may be seen within a few weeks or months of onset.[45] There may be cholestasis. The common finding of steatosis in hepatitis C is discussed in **Chapter 9**. Fulminant hepatitis C is very rare in the Western world,[3] but may be commoner in parts of Asia.[46]

Hepatitis D (delta hepatitis)

Co-infection or superinfection with the hepatitis D virus (HDV) alters the course of type B hepatitis. It encourages chronicity and enhances severity,[47–49] except after liver transplantation. The antigen, HDAg, can easily be demonstrated immunohistochemically in paraffin sections and is mainly found in hepatocyte nuclei (**Fig. 6.17**). These may have finely granular eosinophilic centres (so-called sanded nuclei[50]). Cytoplasmic and membrane-associated staining is also sometimes seen.

Severe acute hepatitis in a patient with markers of HBV infection may be due to superinfection by HDV of a chronic HBV carrier.[51] In an outbreak of HDV infection among Venezuelan Indians, notable features included early small-droplet fatty change, sparse lymphocytes and abundant macrophages in the parenchyma, and substantial portal

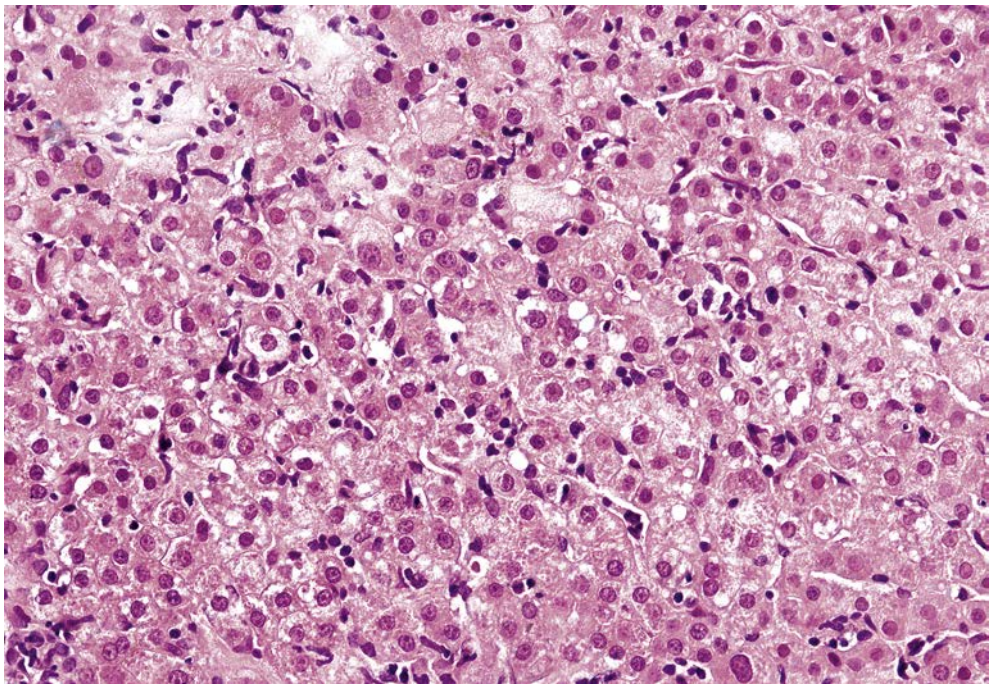

Fig. 6.16 Acute hepatitis C. In this example the main abnormality is infiltration of sinusoids by lymphocytes. (Needle biopsy, H&E.)

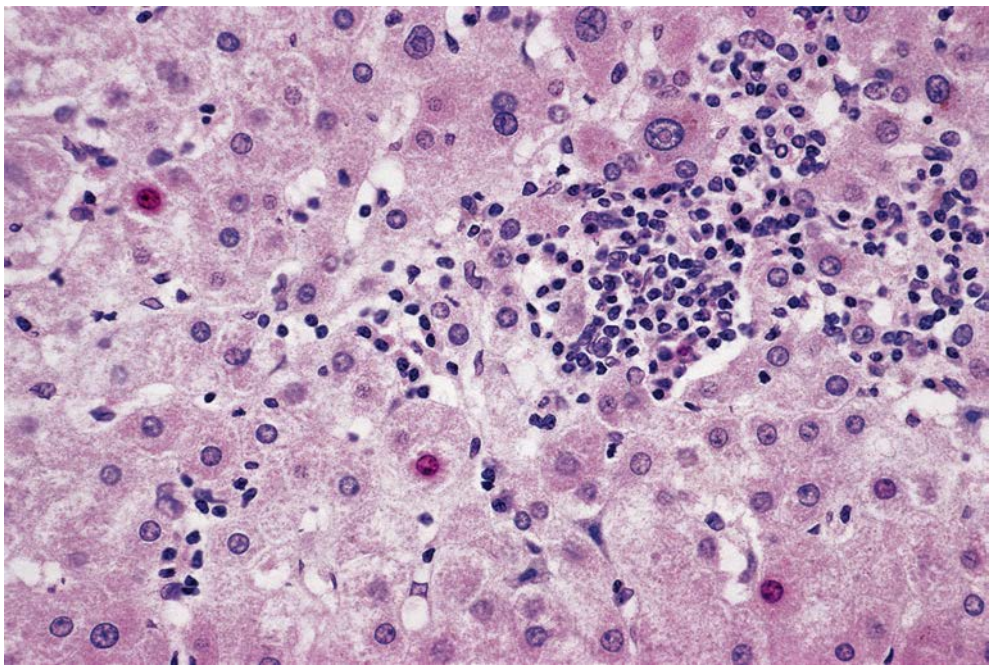

Fig. 6.17 Delta hepatitis (HDV). Some hepatocyte nuclei contain the delta antigen and are stained red. There is a substantial lymphocytic infiltrate. (Needle biopsy, specific immunostain, alkaline phosphatase method.)

infiltration.[52] Later in the attack, there was extensive necrosis and collapse. Microvesicular fatty change and acidophilic necrosis of hepatocytes have been reported from Colombia[53] and North America.[54] In non-immunosuppressed patients with current HDV infection, liver biopsy is likely to show substantial necrosis and inflammation. However, there are HDV-endemic regions where the virus produces little significant disease.[55] Following liver

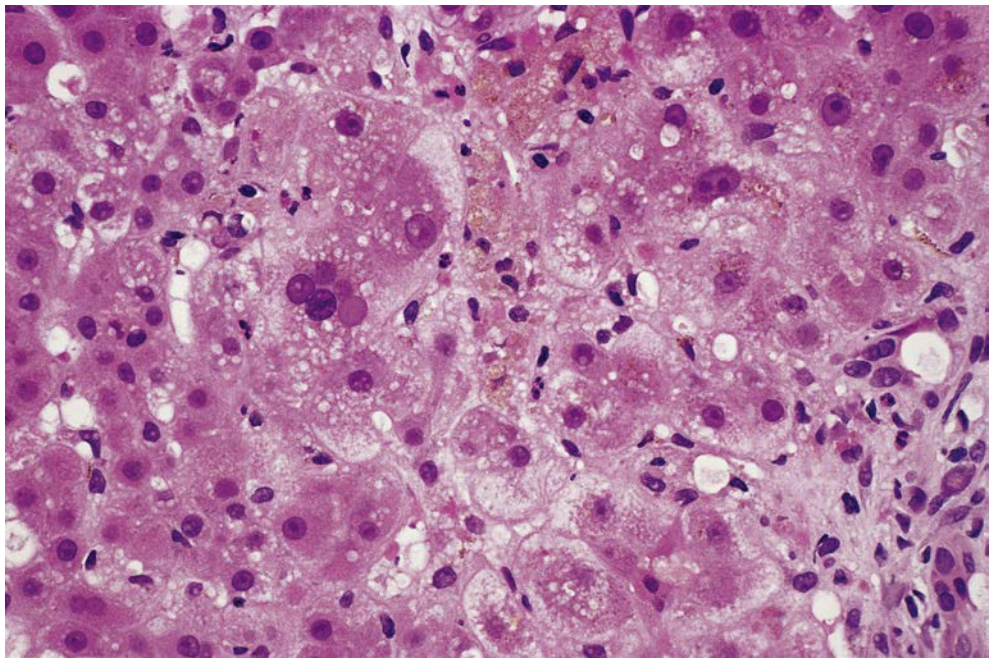

Fig. 6.18 Hepatitis E. Hepatocytes are vacuolated and one to the left of centre is greatly enlarged and multinucleated. There is a mixed infiltrate, and macrophages contain brown ceroid pigment. (Needle biopsy, H&E.)

transplantation, by contrast, HDV without HBV is sometimes demonstrable in the absence of hepatitic changes, indicating that HDV can survive in the absence of HBV. It does not then appear, however, to be capable of causing liver damage.[56]

Hepatitis E virus LBI-10

Hepatitis E virus (HEV) is an RNA virus with eight currently described genotypes, five of which can infect humans (genotypes 1, 2, 3, 4, and 7).[57,58a] Genotypes 1 and 2 are restricted to higher primates and humans and are associated with epidemic outbreaks and an oral-faecal transmission mode, while genotypes 3-8 show a broad mammalian phylogenetic reservoir including pigs, boar, deer, rodents, ferrets, bats, cattle, sheep, foxes, dromedary camels and horses and cause zoonotic, autochthonous (i.e., acquired regionally) infections, typically through poorly or undercooked meat.[58b,58c] HEV has caused epidemics in Asia and has also been found in Africa, North and South America and Europe. Infections with genotypes 1 and 2 resulted in an estimated >3 million symptomatic cases and 70,000 fatalities in endemic regions in 2005.[59a,59b] Autochthonous HEV infections, among which genotype 3 is the most common, have caused acute hepatitis in North America and Europe[57,60] but sometimes is misdiagnosed as drug-induced liver injury.[61a,61b,61c] Chronic hepatitis E has been described in organ transplant recipients and other immunosuppressed individuals.[62,63a,63b]

Information about the pathology of HEV infection in humans is emerging,[58a,61a,63c] although many of the histological features are similar to those seen in other types of viral hepatitis or in autoimmune or drug-induced hepatitis.[58a,61a] The morphology depends on the HEV viral genotype and on the clinicopathological setting[58a,63a-67] (see Box 6.1). Foci of lobular necroinflammation with intrasinusoidal pigmented, ceroid-laden Kupffer cells are prominent in the acute infection (**Fig. 6.18**).[58a] *Epidemic (genotype 1 or 2) hepatitis E* is well known for its potential for severe hepatic disease, acute liver failure and massive hepatic necrosis, but some cases have shown prolonged clinical cholestasis with bile canalicular cholestasis and cholestatic rosettes on biopsy. The changes may resemble those of hepatitis A, with prominent cholestasis

Box 6.1 Major clinicopathological settings of HEV infection

- Acute, epidemic (genotype 1 or 2) hepatitis E
- Acute autochthonous (genotype 3 or 4-7) hepatitis E
- Acute hepatitis E superimposed on pre-existing chronic liver disease (acute-on-chronic liver disease)
- Acute or chronic hepatitis E in immunocompromised host (e.g., organ transplant recipients; chemotherapy administration; HIV positivity)
- Chronic hepatitis E

and a predominantly portal and periportal inflammatory infiltrate.[68] In one study, a pregnant woman with fatal epidemic hepatitis E the liver showed little portal inflammation, much cholestasis with prominent portal vein and central vein endotheliitis and viral particles were identified in bile ductules by electron microscopy.[69] *Cases of autochthonous hepatitis E (usually genotype 3)* have shown portal lymphoid aggregates and periportal ductular reaction with neutrophilia at the edges of portal tracts.60 In immunocompromised subjects with organ transplants, immundeficiencies or corticosteroid therapy, *chronic hepatitis E* manifests with the classical features of chronic hepatitis (i.e., interface hepatitis with variable lobular necroinflammation), but portal tract neutrophilia with bile duct damage, or even destruction, may be prominent.[70]

Detection of HEV infection in liver tissue can be accomplished by polymerase chain reaction (PCR) assessment for HEV RNA, or by immunohistochemistry for open reading frames (ORFs) 1-3 (especially ORF 2) and by in situ hybridization for HEV RNA.[71,72] Recent efforts to produce a vaccine have shown promise, but the only existing vaccine (vaccine 239) is currently only licensed in China.[73-76]

Differential diagnosis of acute viral hepatitis

The distinction of acute hepatitis from bile-duct obstruction rests mainly on the finding of typical hepatitic changes in the parenchyma. The portal tract oedema of duct obstruction is absent. Drug-related hepatitis may be indistinguishable from viral hepatitis and should always be suspected if the cause of the hepatitis is in doubt. Features more common in drug-induced than in viral hepatitis include sharply defined perivenular necrosis, granulomas, bile-duct damage, abundant neutrophils or eosinophils and a poorly developed portal inflammatory reaction. Cholestasis may overshadow the hepatitic features. Autoimmune hepatitis may have a clinically acute onset, histologically indistinguishable from viral hepatitis or alternatively with histological features of chronic disease. This is discussed more fully in **Chapter 9**. In steatohepatitis there is usually conspicuous fatty change. Mallory bodies may be present in ballooned hepatocytes, and the infiltrate typically includes neutrophils. The key to the diagnosis is the presence of pericellular fibrosis in affected areas. The differentiation of acute from chronic hepatitis is briefly discussed under bridging necrosis in **Chapter 4**. While the parenchymal changes predominate in acute hepatitis, especially in perivenular areas, portal and periportal changes predominate in chronic disease. The distinction is sometimes difficult to make, especially when extensive lobular changes are found during an exacerbation of chronic hepatitis or in reactivated chronic hepatitis B, as described earlier.

Fate and morphological sequelae of acute viral hepatitis

Resolution

As far as can be deduced from the available evidence, most examples of hepatitis A, B and E are followed by complete or near-complete resolution and a return of the liver to normal.

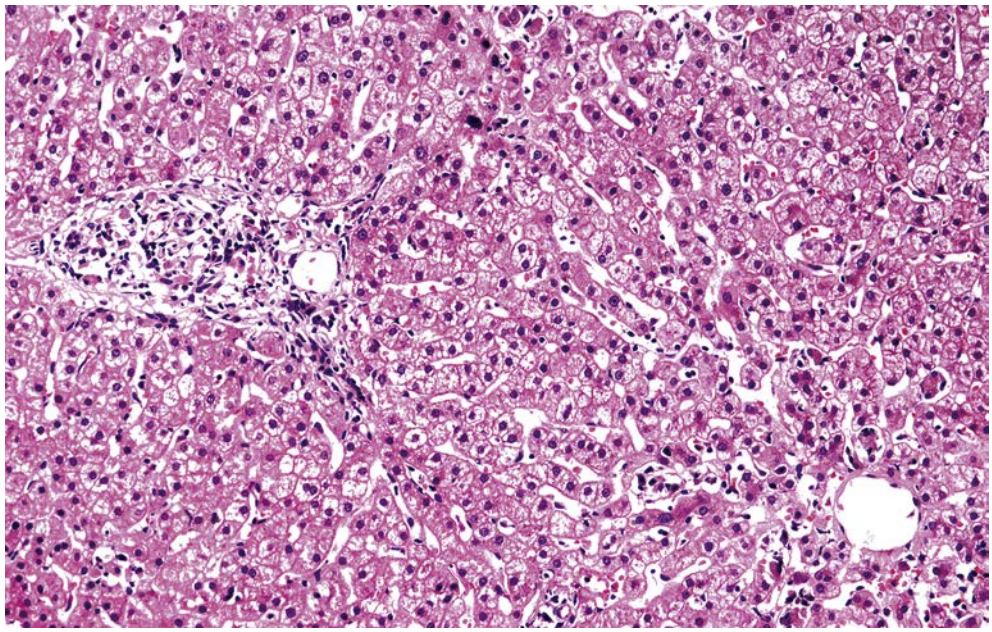

Fig. 6.19 Acute viral hepatitis: residual changes. Short septa extend from the mildly inflamed portal tract to the left. Minimal inflammation and irregular liver-cell plates are seen around the efferent venule below right. (Needle biopsy, H&E.)

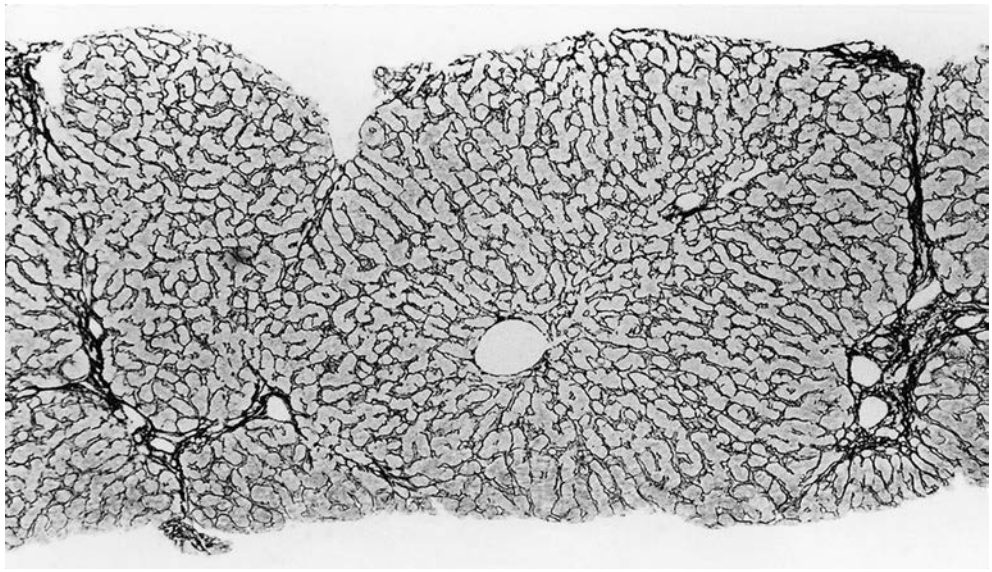

Fig. 6.20 Acute viral hepatitis: residual changes. Slender septa link portal tracts (left and right), but the perivenular area (centre) is unaffected and architectural relationships are preserved. (Needle biopsy, reticulin.)

A chronic course is probably more common when hepatitis B is complicated by delta infection than otherwise, and in hepatitis C the risk of chronicity is high. Even in patients whose hepatitis resolves, some residual changes may persist for many months after clinical recovery (Figs. 6.19 and 6.20).

Scarring

Localised collapse, scarring and regeneration following severe hepatitis with bridging or panlobular necrosis sometimes produce a histological picture indistinguishable from cirrhosis.

Fatal outcome or need for liver transplantation

Necrosis is usually severe. Regenerative hyperplasia of surviving hepatocytes or progenitor cells may be seen.

Chronic hepatitis

In regions where HBV vaccine programs have not been instituted, many individuals will develop chronic hepatitis B. The availability of direct-acting antiviral agents to treat acute hepatitis C virus infections is likely to dramatically reduce the prevalence of chronic hepatitis C in the future.

Cirrhosis

Cirrhosis resulting from infection with a hepatitis virus almost always follows a period of chronic hepatitis, with repeated or continuous hepatocellular necrosis and regeneration. Occasionally it may follow directly after a single episode of severe acute hepatitis where it is termed 'postnecrotic cirrhosis'.

Hepatocellular carcinoma

This may develop on the basis of cirrhosis in patients infected with HBV or hepatitis C virus. Occasionally, however, hepatocellular carcinoma is found in the absence of cirrhosis, usually after a prolonged period of chronic liver disease[76] (although the risk appears to be even greater in non-cirrhotic patients with non-alcoholic steatohepatitis).[77,78]

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CHAPTER

7 Steatosis, Steatohepatitis and Related Conditions

Steatosis

Steatosis (fatty change, fatty liver) is the accumulation of abnormal amounts of lipid in hepatocytes. Most steatosis is of the macrovesicular type, in which a single large fat vacuole or several smaller ones occupy the greater part of the cell, pushing the nucleus to the periphery (Fig. 7.1). The less common and often more serious type is microvesicular steatosis (Fig. 7.2). The fat in this type is finely divided and the nucleus remains central. The two types of steatosis are sometimes found together, though one type usually predominates.

Macrovesicular steatosis

Macrovesicular steatosis is common. It is frequently apparent by non-invasive imaging and may be accompanied by moderate abnormalities of serum aminotransferases, alkaline phosphatase and γ-glutamyl transpeptidase. Liver tests may be normal.[1] There

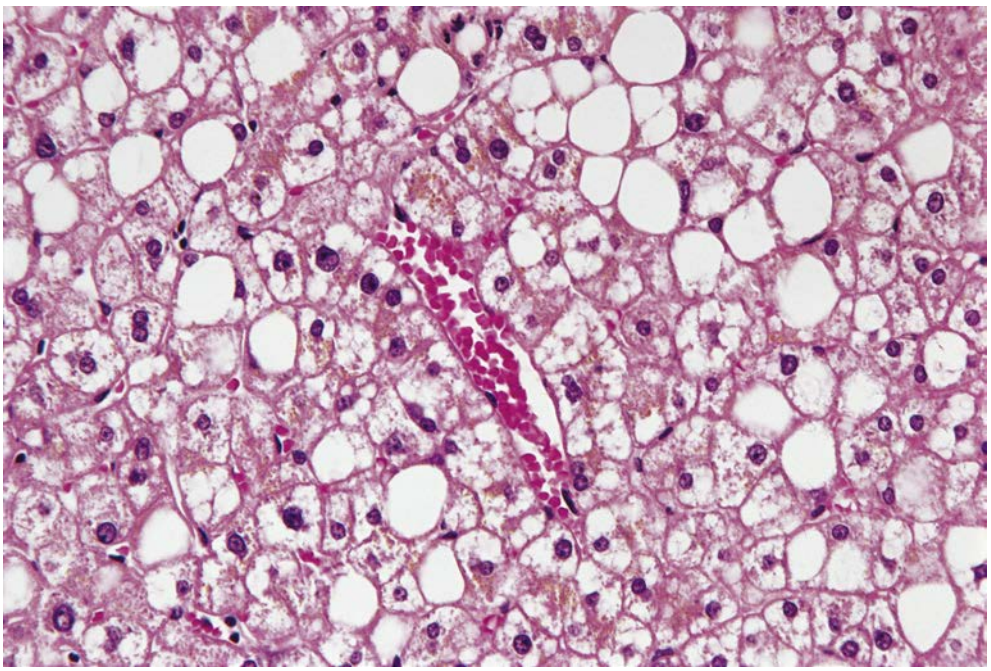

Fig. 7.1 Macrovesicular steatosis. There are large fat vacuoles in perivenular hepatocytes, displacing the nuclei to the edges of the cells. (Needle biopsy, H&E.)

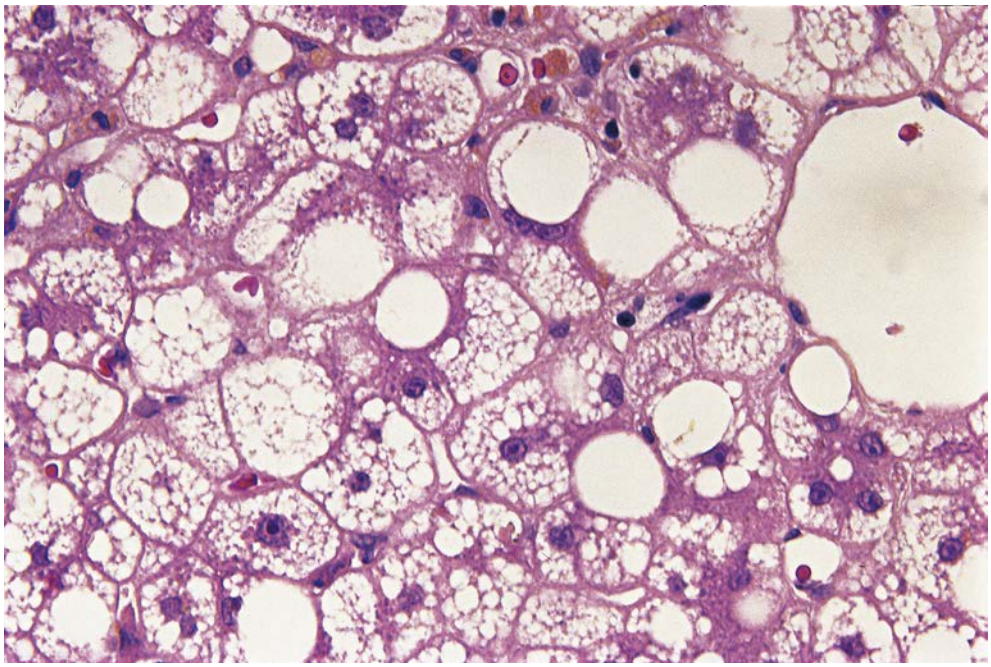

Fig. 7.2 Microvesicular steatosis. Swollen hepatocytes near an efferent venule (right) contain numerous small vacuoles. The nuclei have maintained their central position. Some large fat vacuoles are also present. (Needle biopsy, H&E.)

are many causes of macrovesicular steatosis, of which the most common are listed in **Box 7.1**. It is usually not possible to determine the cause of uncomplicated large-droplet steatosis from histological examination alone.

Box 7.1 Common causes of macrovesicular steatosis

Obesity and diabetes mellitus
Protein-calorie malnutrition
Total parenteral nutrition
Drugs and toxins (e.g. alcohol, corticosteroids)
Metabolic disorders (e.g. Wilson's disease)
Infections (e.g. hepatitis C)

The lipid in macrovesicular steatosis accumulates in hepatocytes because of increased triglyceride synthesis or decreased excretion.[2] Increased synthesis results from availability of excess free fatty acids and fatty acid precursors and from reduced fatty acid oxidation. Reduced excretion is a result of diminished apoprotein production, seen for example in protein malnutrition and alcohol abuse. Current investigations of fatty liver disease indicate that this view is overly simplistic. The pathogenesis of hepatic steatosis is far more complex and is affected by phenotypic variations in enzymes involved in lipid metabolism, in inflammation and in fibrosis, as well as zonal representation of specific enzymes and lipid moieties involved in lipid metabolism (lipid zonation) within the lobule/acinus.[3,4] For example, fatty acid oxidation is at a higher rate in periportal regions than elsewhere, in part at least because of higher content of the oxidative enzyme carnitine palmitoyltransferase-1 in periportal hepatocytes.[4] The spectrum of possible phenotypic variations among individuals is obviously considerable and influences the distribution and type of steatosis and its evolution to steatohepatitis and cirrhosis. For the pathologist, the impact of such data is to serve as a practical reminder that all fatty livers are not the same and that each warrants careful examination for the lobular distribution of steatosis, the type of steatosis (i.e. macro- vs microvesicular) and any unusual associated histological features (**Figs 7.3 and 7.4**).

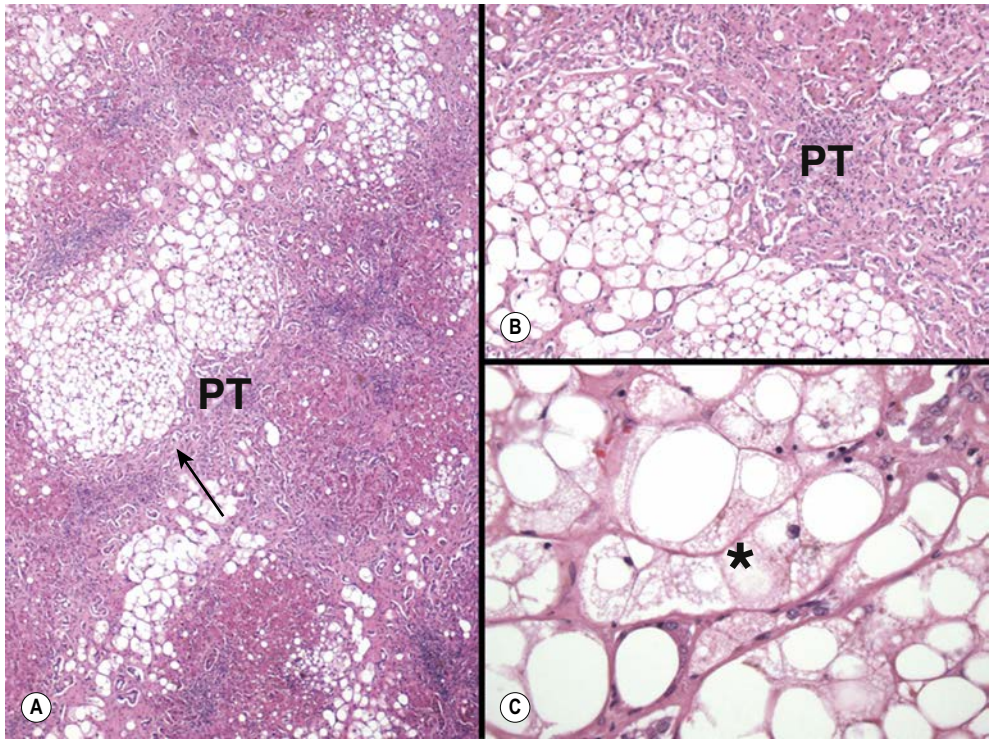

Fig. 7.3 Zonation of steatosis with periseptal and periportal prominence in cirrhosis due to obesity and non-alcoholic steatohepatitis. A: Macrovesicular steatosis is prominent in hepatocytes near the portal tract (PT). The portal and periportal fibrosis is associated with a conspicuous ductular reaction (arrow). **B:** Hepatocytes adjacent to the PT contain lipid vacuoles of varied size. **C:** The periportal 'zonation' of steatosis with storage of heterogeneous sizes of lipid vacuoles in hepatocytes is related to the known zonal distribution of lipid metabolic enzymes such as carnitine palmitoyltransferase-1 (localised to periportal hepatocytes)[4]. Note that despite the absence of significant inflammation in this microscopic field, the presence of the swollen hepatocytes with rarefied, wispy cytoplasm (*) is sufficient evidence of steatohepatitis. (Explant liver, H&E).

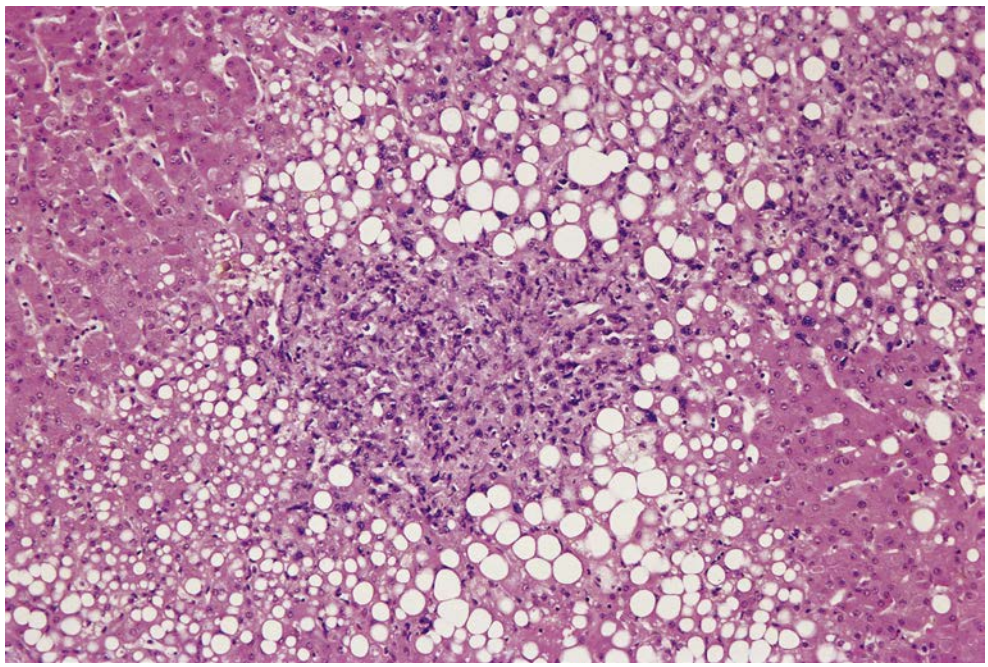

Fig. 7.4 Periportal steatosis. Liver biopsy from a patient with acquired immunodeficiency syndrome and portal tract infiltration by large-cell lymphoma. Periportal hepatocytes contain large fat vacuoles. (Needle biopsy, H&E.)

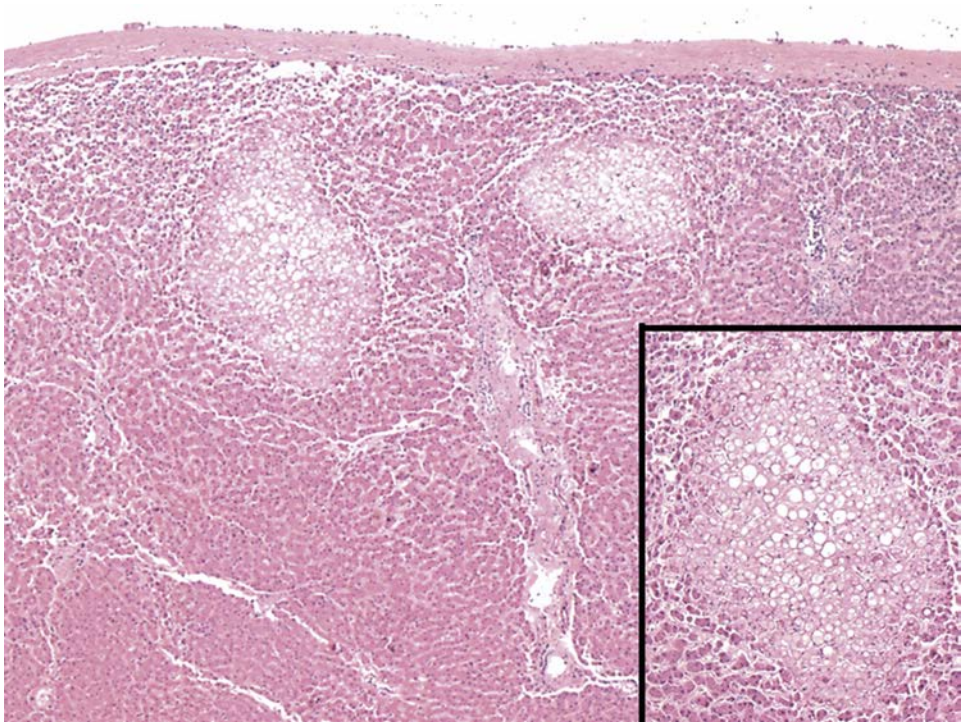

Fig. 7.5 Focal fat. Two subcapsular foci of focal fat in an otherwise non-steatotic liver are present. Inset: Focal fat is typically macrovesicular. (Post-mortem liver, H&E).

Macrovesicular steatosis provides the background on which the important lesions of alcoholic and non-alcoholic steatohepatitis (NASH) develop.[5] Moreover, macrovesicular steatosis is increasingly being recognised as a significant risk factor for hepatocellular carcinoma, even without preceding fibrosis or cirrhosis, particularly when widely prevalent risk factors for metabolic syndrome such as obesity and diabetes are present.[6–11] Most steatosis is **perivenular** (centrilobular regions/acinar zones 3). Alcohol use, adult obesity, diabetes and corticosteroid therapy typically show this location. Increasing amounts of steatosis extend to progressively involve mid-zonal and periportal regions (acinar zones 2 and 1, respectively). With increasing amounts of macrovesicular fat there sometimes are interspersed clusters or patches of hepatocytes with microvesicular steatosis,[12] probably reflecting the evolution of large lipid vacuoles from progressive coalescence of small lipid droplets.[13,14] By contrast, **periportal steatosis** is more common in children with non-alcoholic fatty liver disease (NAFLD; discussed later), in patients on parenteral nutrition, in kwashiorkor and protein-calorie malnutrition, and it is sometimes seen in acquired immunodeficiency syndrome (AIDS; **Fig. 7.4**). In **focal fatty change**[15] more or less rounded foci of steatosis are seen in an otherwise normal liver and may be mistaken for neoplasms on imaging (**Fig. 7.5**). In many cases the cause is unknown, but exposure of the capsular surface of the liver to insulin (e.g. in diabetics receiving peritoneal dialysis and intraperitoneal insulin) or in liver tissue adjacent to a metastatic insulinoma the lesion may become apparent radiologically.[16]

The histological **grade** of steatosis should be reported based on the percentage of hepatocytes which contain lipid vacuoles. One commonly used scoring system includes grades of minimal (<5%), mild (5%–30%), moderate (30%–60%) and marked (>60%). Provision of a numerical assessment to the nearest percentile is also recommended (e.g. 'marked macrovesicular steatosis is present involving approximately 90% of the parenchyma'). Periodic acid–Schiff and trichrome stains can be helpful in the assessment, providing contrast of the large lipid vacuoles against the more darkly stained background

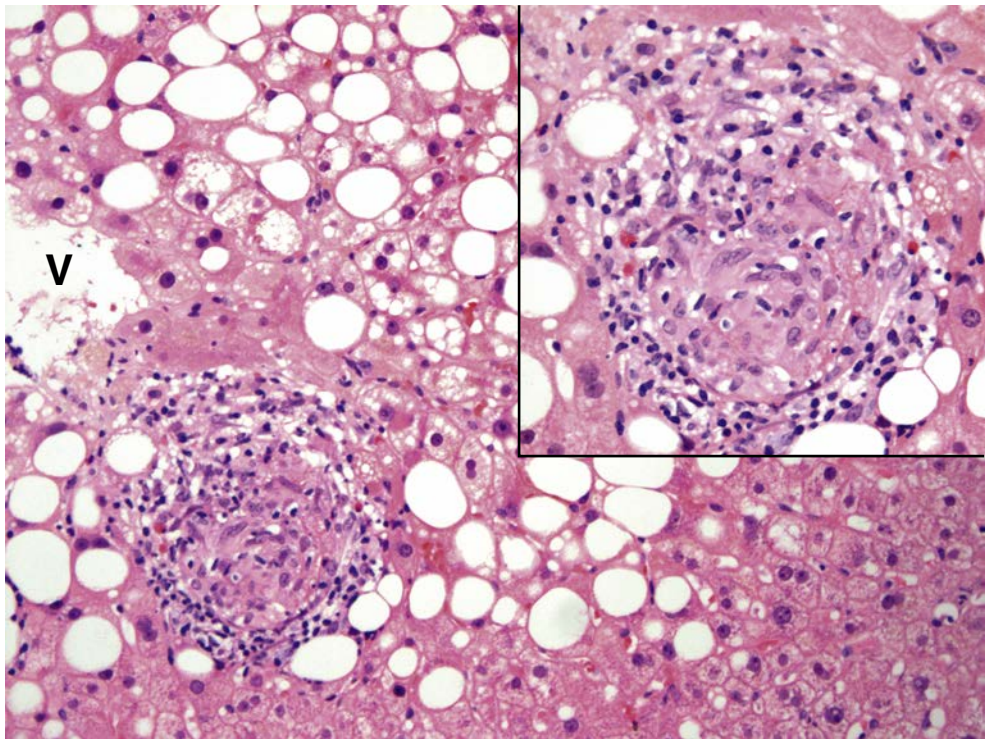

Fig. 7.6 Lipogranuloma. A lipogranuloma has formed near the terminal venule (V) in this moderately steatotic liver. Inset: The lipogranuloma contains large lipid vacuoles and aggregated Kupffer cells with scattered lymphocytes and a few eosinophils. (Needle biopsy, H&E.)

cytoplasm of hepatocytes. Digitised computer image analysis[17,18] is an alternative method of grading but from a practical standpoint is better suited to research settings.[19]

Occasionally lipid-laden hepatocytes rupture and the fat is then taken up by macrophages. The resulting lesion is a **lipogranuloma** (Fig. 7.6). Lipogranulomas are situated within the lobules, often near terminal venules. Serial sectioning may be needed to identify the fat in the centre of the lesion. Lipogranulomas may undergo fibrosis, but this does not appear to contribute to progressive liver disease and must be distinguished from the more important pericellular fibrosis characteristic of steatohepatitis (discussed later). Globules within portal tracts are usually the result of uptake of ingested or injected mineral oils by macrophages, rather than uptake of lipids[20] (Fig. 7.7). **Lipopeliosis**—the formation of large intrasinusoidal fat cysts following release of lipid from hepatocytes after transplantation—is described in Chapter 16.

The differential diagnosis of macrovesicular steatosis includes microvesicular steatosis. The presence of several fat vacuoles in one hepatocyte has to be distinguished from true microvesicular steatosis (see the following section) in which vacuoles are generally less than 1 μm in diameter and may even be invisible in paraffin sections by light microscopy. The distinction is clinically important. The location of the nucleus helps to differentiate the two conditions. A second differential diagnosis is from **stellate-cell hyperplasia** (Fig. 7.8), in which the vacuoles are not in hepatocytes but in perisinusoidally located stellate cells.[21] Their nuclei are compressed into a crescentic shape by the vitamin A-rich globules. Stellate-cell hyperplasia may be unexplained, but should lead to investigation of possible overuse of vitamin A or other retinoids.

Microvesicular steatosis

In this serious and sometimes fatal condition, finely divided fat accumulates in hepatocyte cytoplasm as a result of mitochondrial damage leading to impaired β-oxidation.[22] Causes include acute fatty liver of pregnancy (Ch. 15), hepatotoxic drugs such as valproate and

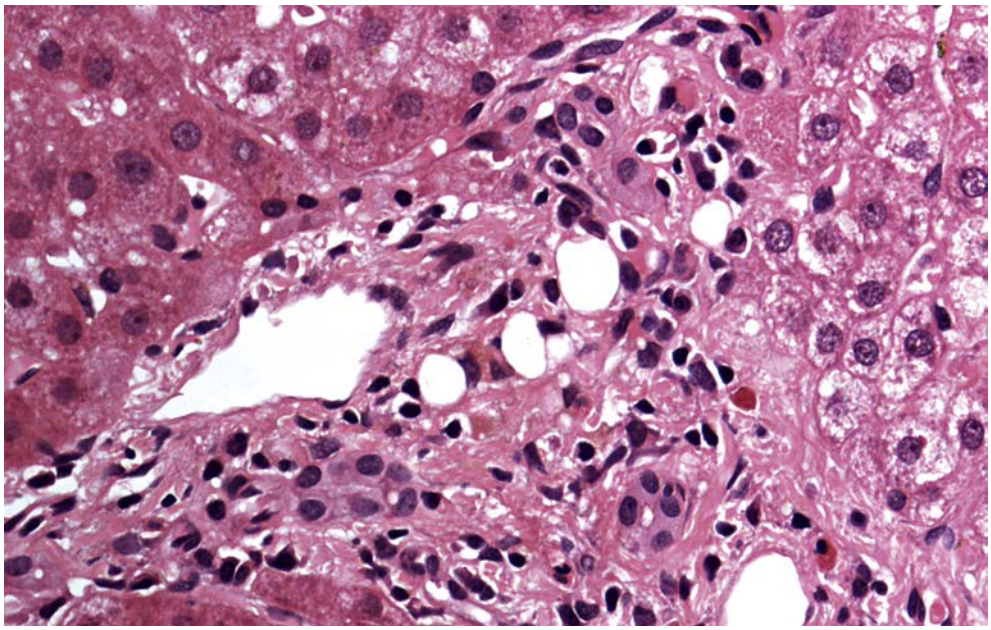

Fig. 7.7 Mineral oil globules. A row of vacuoles within macrophages is seen to the right of a portal venule. (Needle biopsy, H&E.)

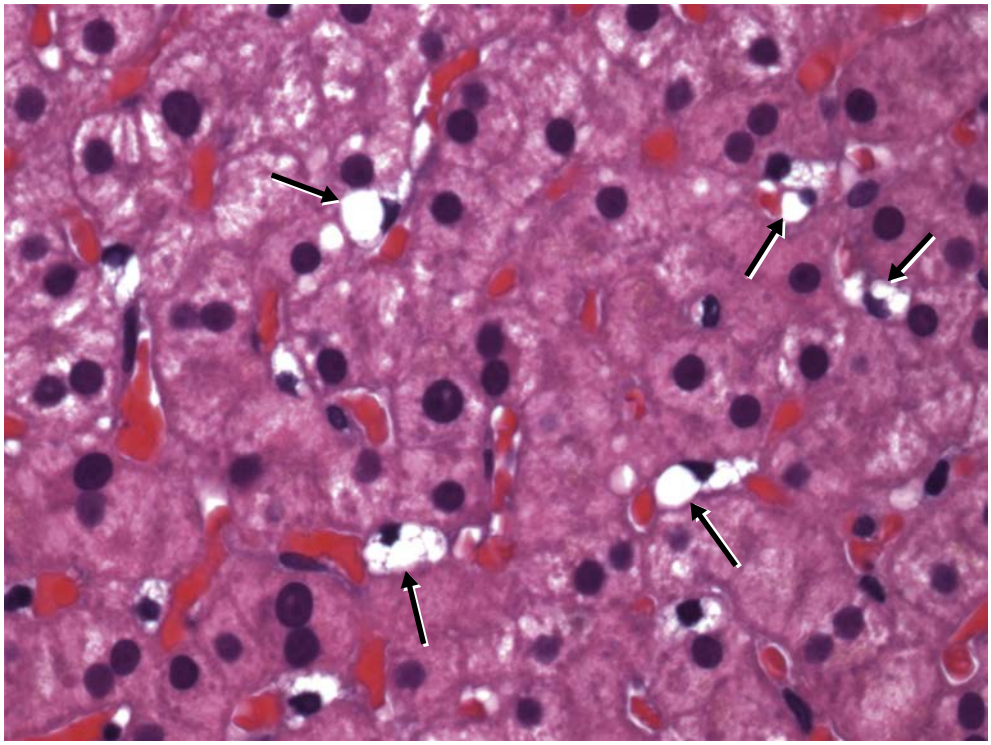

Fig. 7.8 Stellate-cell hyperplasia. Stellate cells with single or multiple lipid vacuoles lie in the space of Disse between hepatocytes (arrows). Stellate-cell nuclei are small, intensely basophilic and indented by the cytoplasmic vacuoles. Hepatocyte nuclei are larger, less dense and rounded. (Needle biopsy, H&E.)

nucleoside analogues (Ch. 8), mitochondrial DNA depletion and deletion syndromes,[23] foamy degeneration in the alcoholic (discussed later) and total parenteral nutrition (Box 7.2). Another cause, Reye's syndrome, has declined sharply in incidence in recent years. In neonates and children, mitochondrial hepatopathies may need consideration.[23–25] Viral infections occasionally give rise to similar changes.[26]

Box 7.2 Main causes of microvesicular steatosis
Acute fatty liver of pregnancy
Alcoholic foamy degeneration
Drugs (e.g. nucleoside analogues, valproate)
Toxins (e.g. in Jamaican vomiting disease)
Total parenteral nutrition
Inborn errors of metabolism (e.g. urea cycle disorders)
Reye's syndrome
Infections

Histologically, the cytoplasmic lipid is seen to be very finely divided and is not always obvious in paraffin sections. It can be stained with oil red O in frozen sections. The affected hepatocytes are often swollen. Their nuclei remain central (**Fig. 7.2**).

The differential diagnosis is from macrovesicular steatosis and from conditions in which hepatocytes are swollen for other reasons, such as hepatitis. As discussed in **Chapter 13**, phospholipids and sphingolipids accumulate in various metabolic disorders. Cholesterol esters accumulate in hepatocytes in Wolman's disease and cholesterol ester storage disease, and glycogen accumulates in glycogen storage disease and diabetics with glycogenic hepatopathy (discussed later).

It bears noting that the terms 'macrovesicular steatosis' and 'microvesicular steatosis' are preferable for use (in lieu of the colloquial 'macrosteatosis' and 'microsteatosis').

Alcoholic and non-alcoholic fatty liver disease

The terms **alcoholic fatty liver disease** (AFLD) and **non-alcoholic fatty liver disease** (NAFLD) are used to describe the complete range of changes from uncomplicated macrovesicular steatosis to steatohepatitis and cirrhosis seen in alcohol abuse and in obesity, diabetes, hyperlipidaemia and the metabolic syndrome, respectively. Insulin resistance, central (truncal) obesity, type 2 diabetes, hyperlipidaemia and systemic hypertension constitute the *metabolic syndrome*. NAFLD is considered the hepatic expression of the metabolic syndrome.[27,28] The wide prevalence of obesity and diabetes in industrialised countries and in other populations has brought NAFLD to increased attention in clinical and basic science. In the United States, NAFLD is currently the leading cause of abnormal serum aminotransferases and chronic liver disease.[29,30] A similar impact is likely in other Western countries and in other populations where the risk factors for NAFLD are prevalent. Emphasis on the histological evaluation of macrovesicular steatosis and related changes in liver biopsy, explant and postmortem specimens has consequently grown.

Systematic histological approach to macrovesicular fatty liver disease

Histological evaluation in AFLD and NAFLD should take into account not only the presence of large-droplet steatosis, but also evidence of hepatocellular damage, inflammation, fibrosis and siderosis which may also be present. The diagnosis of steatohepatitis should be rendered based on specific histological criteria (described in detail later). In AFLD and NAFLD there may be relatively inconspicuous *apoptotic bodies*.[31–33] On the one hand, increased necroinflammatory activity in NASH may be accompanied by numerous apoptotic bodies.[34] *Focal lobular inflammation* (usually clusters of lymphocytes and activated Kupffer cells) may be seen (**Fig. 7.9**) but does not constitute steatohepatitis. *Hepatocyte ballooning*, on the other hand, is a major feature of both early and of well-developed steatohepatitis (**Figs 7.10 and 7.11**), for which careful inspection is warranted. Ballooned hepatocytes are often identifiable even at low magnification (**Fig. 7.10**). They show watery and oedematous, wispy and rarefied cytoplasm (**Fig. 7.11**). A variety of factors cause this type of ballooning, including perturbed metabolic pathways,[35] cytoskeletal damage[36] (particularly of keratins 8 and 18) and endoplasmic reticulum stress.[37] These ballooned hepatocytes appear to be moribund but yet 'undead', still capable of producing a variety of factors such as Sonic hedgehog which exerts both paracrine and autocrine effects.[38,39] Combination

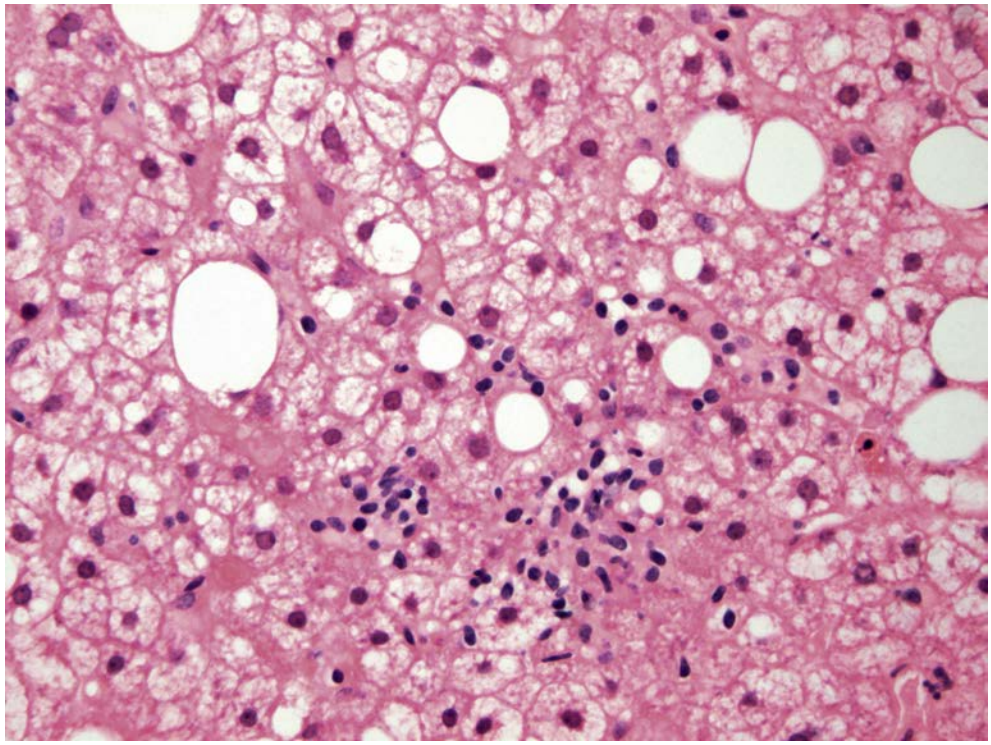

Fig. 7.9 Macrovesicular steatosis with focal inflammation. Scattered lymphocytes are present in this mildly fatty liver. This type of inflammation is relatively common but does not constitute steatohepatitis. (Needle biopsy, H&E).

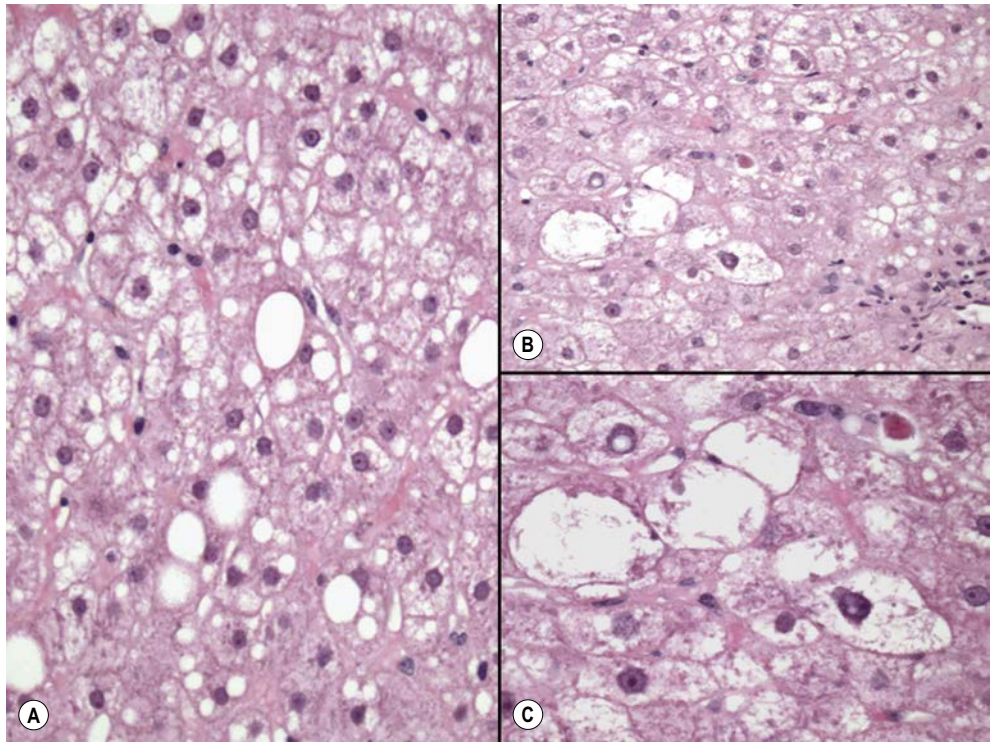

Fig. 7.10 Hepatocyte ballooning in macrovesicular fatty liver disease. A: This liver shows minimal steatosis but no hepatocyte ballooning. Note the uniform size of hepatocytes in this panel, some of which (at upper left) show pale, glycogen-containing cytoplasm but no evidence of ballooning. **B:** A cluster of ballooned hepatocytes is evident at relatively low magnification. **C:** Ballooned hepatocytes are enlarged and show oedematous, wispy and rarefied cytoplasm. Ballooning of this type reflects significant hepatocellular damage, which is an important component of steatohepatitis, and the specimen should be examined carefully for frank steatohepatitis elsewhere. (Needle biopsy, H&E.)

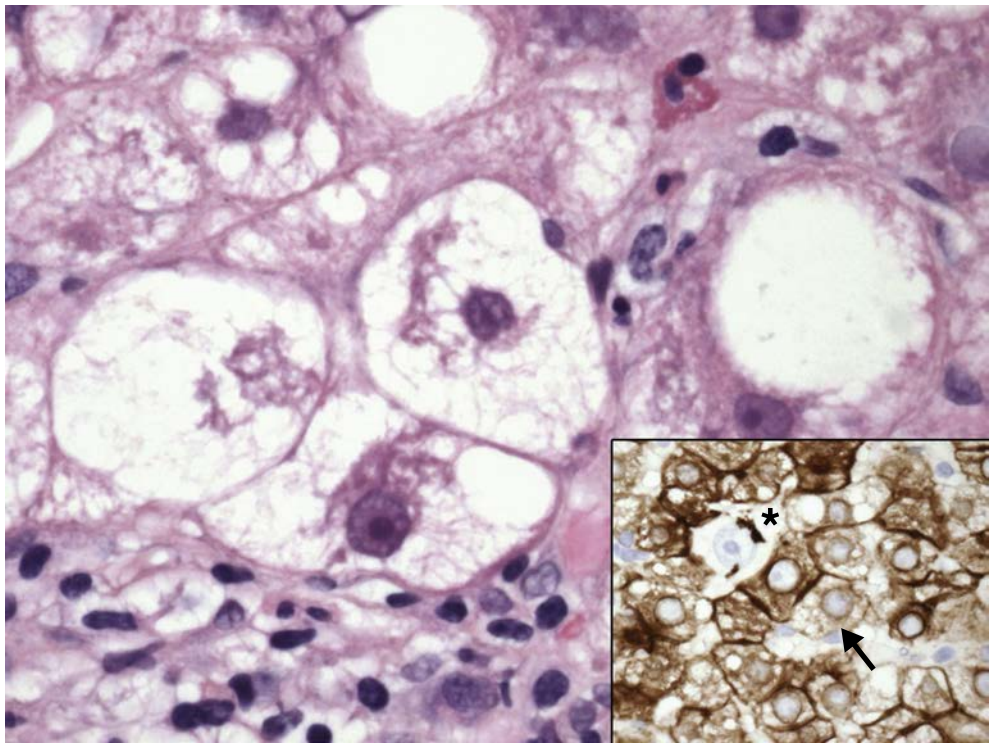

Fig. 7.11 Hepatocyte ballooning in steatohepatitis. The three conspicuously ballooned hepatocytes at the centre of this field have oedematous, wispy and rarefied cytoplasm as well as clumped eosinophilic filamentous material (Mallory–Denk bodies). (Needle biopsy, H&E.) Inset: Combined immunohistochemical stain for cytokeratins 8 and 18 shows absent keratins in several hepatocytes at centre (arrow), one of which (*) contains several darkly stained Mallory–Denk bodies near the nucleus. (Needle biopsy, H&E. Inset: specific immunohistochemistry.)

immunostaining for cytokeratins 8 and 18 (CK8/18) helps to demonstrate affected cells which show either absent or decreased cytoplasmic staining[36,40] (**Fig. 7.11, inset**). The most fully developed histopathological picture of steatohepatitis (**Fig. 7.12**) includes macrovesicular steatosis, hepatocyte ballooning, inflammation, intracellular Mallory–Denk bodies and perivenular fibrosis (discussed in detail later).

Uncomplicated steatosis in the majority of cases is not associated with significant portal tract inflammation. However, focal *minimal or mild portal lymphocytic infiltrates* are sometimes present in either steatosis or steatohepatitis.[41,42] More active cases of NASH with advanced fibrosis sometimes present diagnostic difficulties because of substantial chronic portal inflammation (including lymphoid aggregates), which may raise the possibility of chronic hepatitis as an alternative diagnosis.[42] Any histological doubt on this issue should be resolved by discussion with the clinician and investigations to exclude causes of chronic hepatitis when necessary. Some adult and paediatric patients with NAFLD show positive serum *anti-nuclear and/or anti-smooth-muscle antibodies*,[43–45] raising a clinical suspicion of autoimmune hepatitis (AIH). However, these autoantibodies are usually low-titre and are considered non-specific. The characteristic portal lymphoplasmacytic inflammation, interface hepatitis and regenerative rosettes of AIH are typically absent in the majority of such cases. Rarely, anti-mitochondrial antibody may be positive.[46,47] The pathologist should therefore be alert to this common diagnostic problem of NAFLD with autoantibodies, and recognise that changes of AIH are not usually present.[48,49]

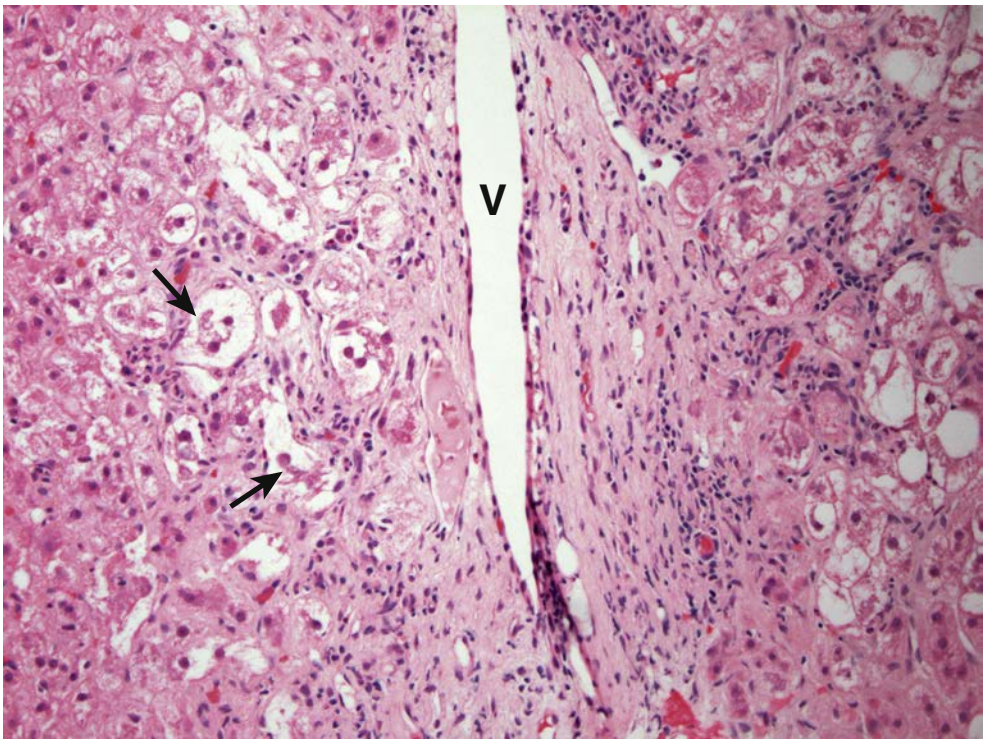

Fig. 7.12 Non-alcoholic steatohepatitis. The terminal venule (V) is surrounded by fibrosis and inflammation. Perivenular hepatocytes are swollen and show cytoplasmic Mallory–Denk bodies (arrows). (Needle biopsy, H&E.)

A connective tissue stain is important to evaluate the extent of any fibrosis and its distribution in the steatotic liver. When there is fibrosis in AFLD and NAFLD, it is usually present as a feature of steatohepatitis and is seen in centrilobular regions (acinar zone 3), as will be described later. Alternatively (and less frequently), there may be portal and periportal fibrosis accompanied by chronic inflammation (**Fig. 7.13**). This distribution is more common in paediatric NAFLD[50] and in morbidly obese individuals.[51–53]

An iron stain should also be reviewed for *siderosis*. Mild iron overload in Kupffer cells and/or hepatocytes may be seen in alcoholic patients because of altered intestinal iron absorption and in up to one-third of individuals with NAFLD due to **dysmetabolic iron overload syndrome (DIOS)**[54–60] (**Fig. 7.14**). Iron overload in this setting increases oxidative stress and hepatocyte apoptosis.[61] Significant hepatocellular siderosis should always prompt consideration of possible primary (genetic) iron overload.

Several systems exist to assess steatosis, inflammation and hepatocellular damage in NAFLD.[62–64] Matteoni and colleagues[63] characterised the spectrum of NAFLD according to four subtypes: NAFLD subtype 1 (simple steatosis), NAFLD subtype 2 (steatosis with inflammation), NAFLD subtype 3 (steatosis with hepatocellular ballooning degeneration; **Fig. 7.10**) and NAFLD subtype 4 (NASH; **Fig. 7.12**). (Subtypes 3 and 4 are now both considered NASH.)[62] Increased morbidity and mortality were associated with types 3 and 4. The NAFLD Activity Score (NAS)[64] is used to determine the presence of NASH and is discussed later (see the 'Non-alcoholic steatohepatitis' section). Similar scoring systems have not been available for AFLD, although a recently proposed system utilises histological features (degree of fibrosis, degree of neutrophil infiltrates, type of cholestasis and presence of megamitochondria) to predict 90-day mortality.[65] Ultimately, the choice and use of a scoring system vary among pathologists and institutions and the

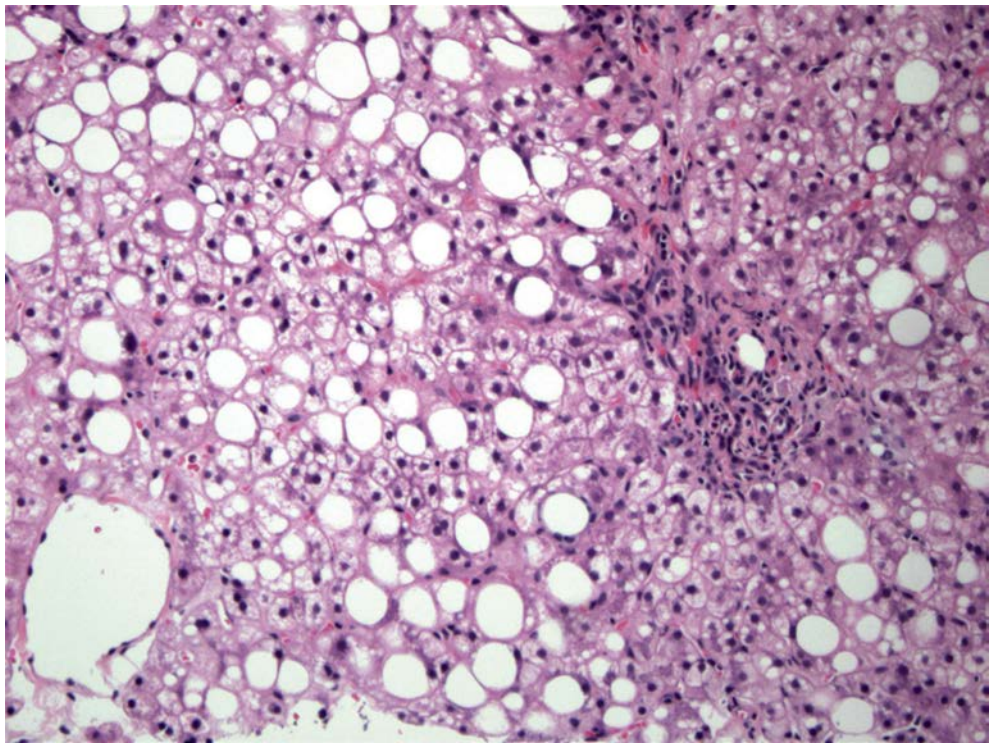

Fig. 7.13 Steatosis with mild periportal fibrosis and chronic inflammation (non-alcoholic steatohepatitis (NASH) type 2). This biopsy from an obese child shows large-droplet steatosis with mild periportal fibrosis and chronic inflammation (at right). This pattern in children with non-alcoholic fatty liver disease is referred to as NASH type 2. Note the absence of liver-cell ballooning, Mallory–Denk bodies, inflammation or fibrosis near the terminal venule at lower left. (Needle biopsy, H&E.)

system(s) used may be selected for specific clinical and research needs. At minimum, though, the pathologist needs to be able to determine when steatohepatitis is present and what degree of fibrosis, if any, has developed, because these features have impact on therapy and prognosis.

Diabetes mellitus

In patients with diabetes mellitus, glycogen vacuolation of hepatocyte nuclei is common[66] (**Fig. 7.15**). These 'glycogen nuclei' are also seen in Wilson's disease (**Ch. 14**), in NASH and in biopsies from children and adolescents less than 14–15 years of age. Hepatomegaly in diabetic patients is not always attributable to steatosis: rarely, patients whose diabetes is poorly controlled may develop Mauriac's syndrome,[67] with abnormal serum liver tests and massive accumulation of glycogen in hepatocytes (**glycogenic hepatopathy**[68]), giving rise to a picture closely resembling inherited glycogen storage disease (**Fig. 7.16**). Some of these patients have mild periportal fibrosis similar to that seen in glycogen storage disease.[69] Some diabetic patients with **diabetic hepatosclerosis**[70] (**Fig. 7.17**) show an increase in perisinusoidal type IV collagen[71] without a zonal predilection. Vascular disease involving hepatic arterioles, causing **hyaline arteriolosclerosis** (with visible intramural hyaline deposits evident on Periodic acid–Schiff stain), is an additional lesion in this population, many of whom also have hypertension.[72]

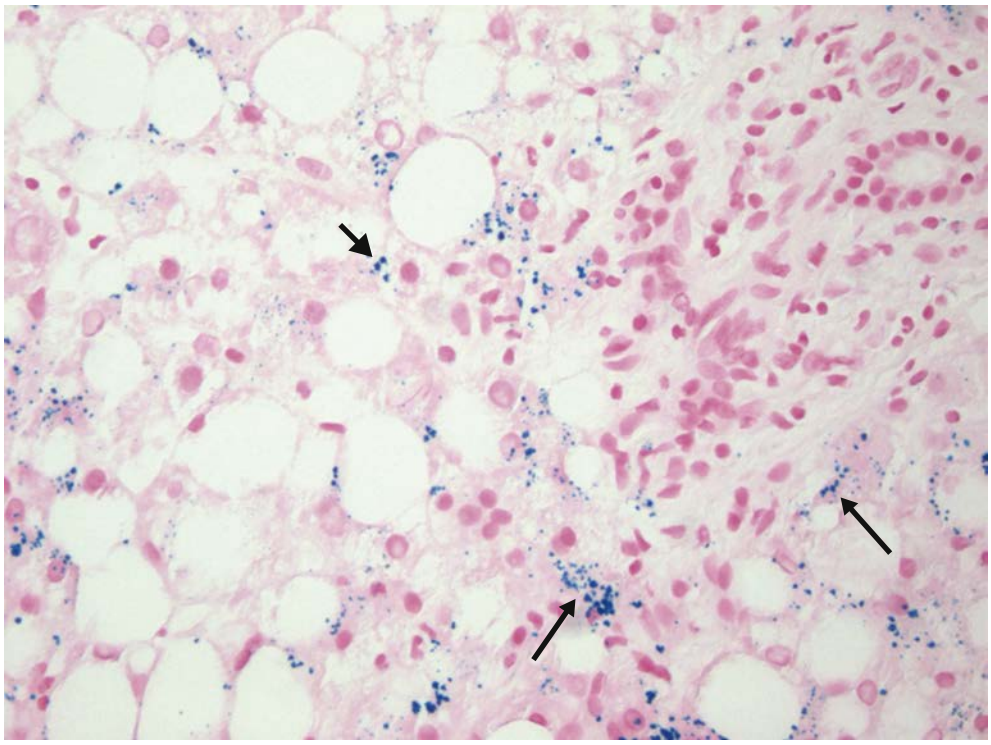

Fig. 7.14 Dysmetabolic iron overload syndrome. This case of non-alcoholic fatty liver disease with marked steatosis is associated with mild siderosis of periportal hepatocytes (long arrows) and sinusoidal Kupffer cells (short arrow). (Needle biopsy, Prussian blue iron stain.)

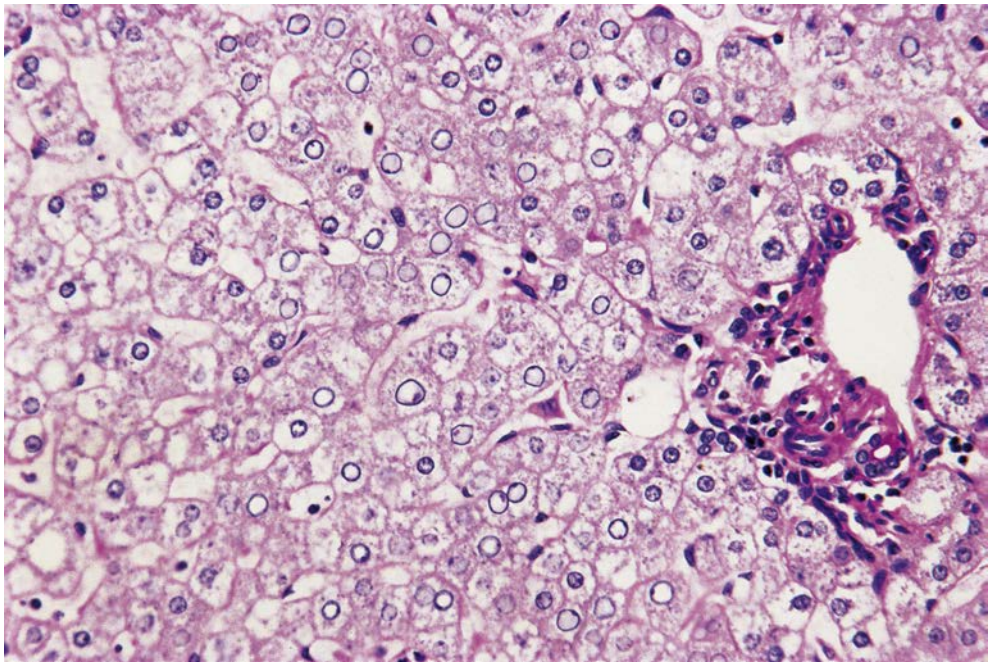

Fig. 7.15 Diabetes mellitus. Glycogen vacuolation is seen in the nuclei of most periportal hepatocytes. (Needle biopsy, H&E.)

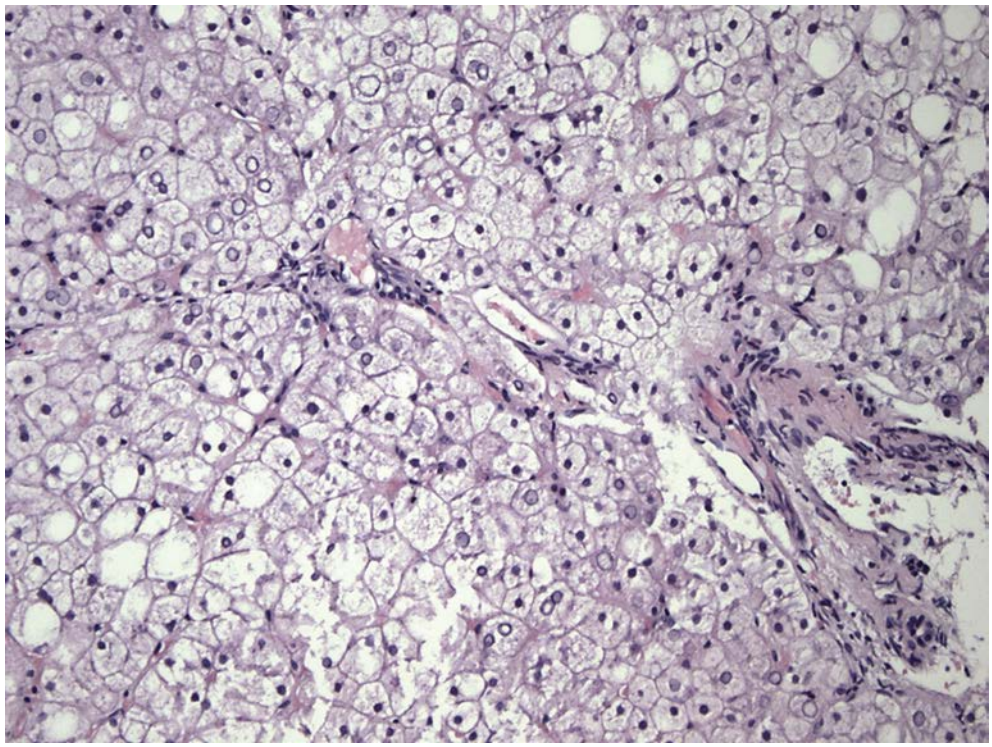

Fig. 7.16 Mauriac syndrome with glycogenic hepatopathy. Enlarged hepatocytes with abundant glycogen stores appear pale and have thickened cell membranes which resemble plant cell walls. The features are similar to those of glycogen storage disease. The subject was a poorly controlled diabetic with abnormal serum liver tests and hepatomegaly (note the diabetes-related 'glycogen nuclei' in periportal hepatocytes at upper left). (Needle biopsy, H&E.)

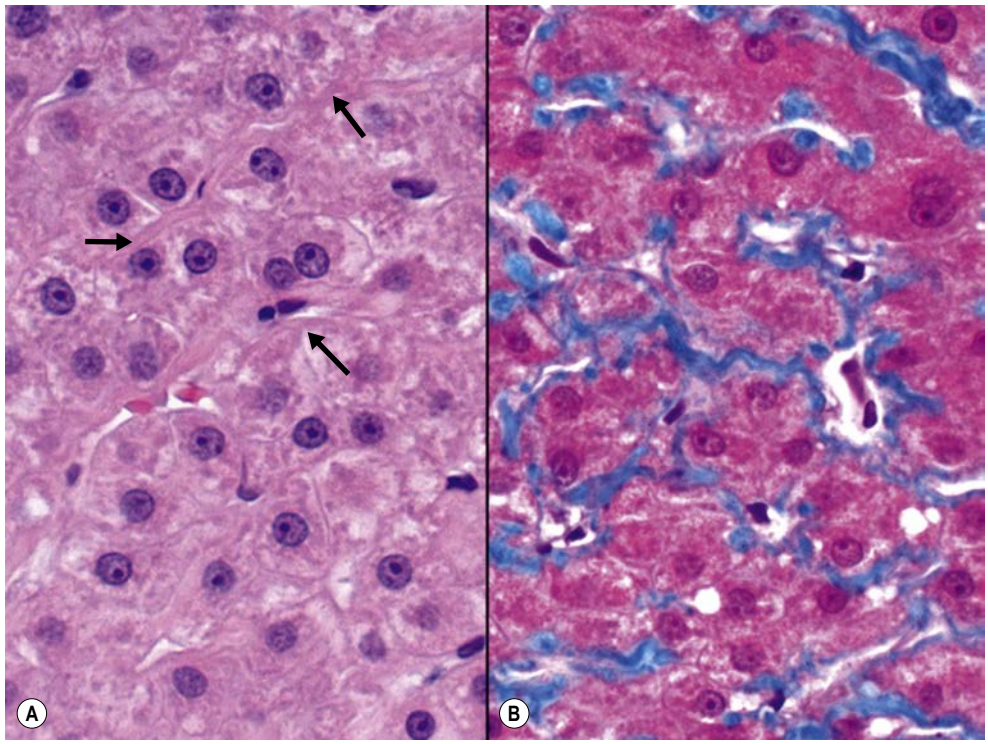

Fig. 7.17 Diabetic hepatosclerosis. A: Increased perisinusoidal collagen is evident (arrows). (H&E.) **B:** The increased perisinusoidal collagen shows no zonal preference. (Trichrome stain.) (Photomicrographs kindly provided by Dr Elizabeth Brunt, St Louis, MO, USA.)

Steatohepatitis, alcoholic and non-alcoholic

In some patients with steatosis an inflammatory and fibrosing lesion, steatohepatitis, develops. This may then lead to cirrhosis. Some patients later develop hepatocellular carcinoma. Most patients with steatohepatitis are alcohol abusers or are overweight, diabetic or have a combination of attributes of the metabolic syndrome. The terms *alcoholic steatohepatitis* (ASH) and *non-alcoholic steatohepatitis* (NASH) are used accordingly. In a minority of patients NASH is associated with other factors, listed later in this chapter. The risk of developing steatohepatitis and cirrhosis in the alcoholic group rises with the amount of alcohol consumed daily,[73a] but genetic and other factors are also influential. Although simple steatosis in individuals with NAFLD has previously been considered a clinically benign and non-progressive condition, recent studies indicate the potential over time for progression to steatohepatitis, cirrhosis and hepatocellular carcinoma.[6–8] Some individuals with NAFLD and hepatocellular carcinoma are non-cirrhotic and have either no fibrosis or more advanced stages of fibrosis.[73b]

Box 7.3 Main pathological features of steatohepatitis

Steatosis
Hepatocyte ballooning
Hepatocyte apoptosis
Mallory body formation
Inflammatory infiltration
Neutrophils
Lymphocytes
Sinusoidal cells
Fibrosis
Pericellular
Other

Pathological features of steatohepatitis

The changes in ASH and NASH are very similar, and the two conditions cannot usually be distinguished on histological grounds alone.[74] The main pathological features comprise hepatocellular damage, inflammation and fibrosis (**Box 7.3**). The following description is of the fully developed lesion.

Hepatocellular damage is generally most severe in, or even restricted to, perivenular areas (**Fig. 7.18**). It takes the form of cell swelling and clearing of the cytoplasm, together with the appearance of Mallory–Denk bodies (Mallory bodies, Mallory's hyalin[75]). The affected

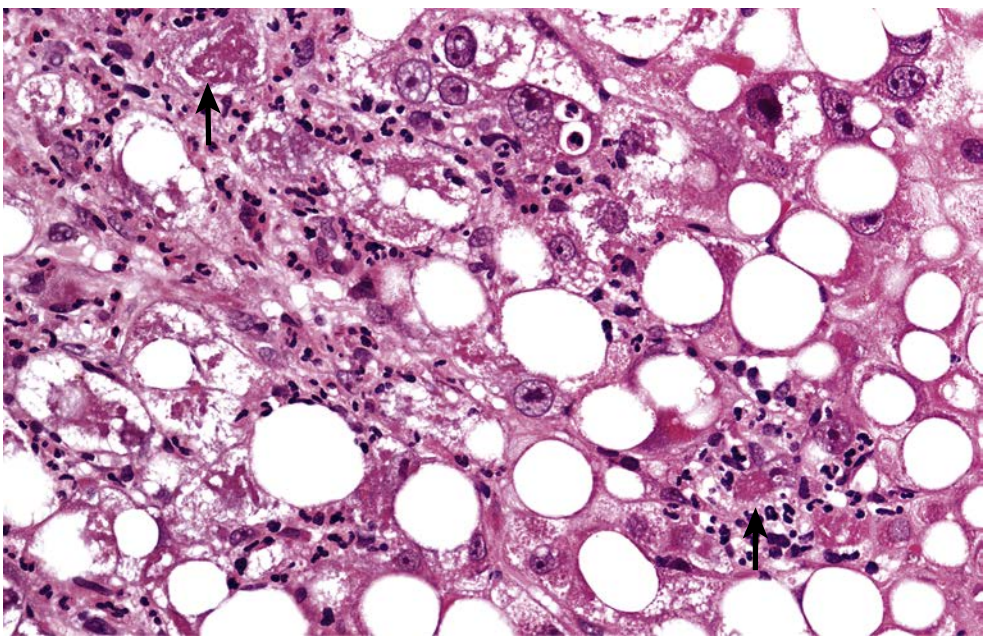

Fig. 7.18 Alcoholic steatohepatitis. Inflammatory cells, mainly neutrophils, are clustered around and within hepatocytes, some of which contain densely stained Mallory bodies (arrows). Many hepatocytes contain large fat vacuoles. (Needle biopsy, H&E.)

cells often do not contain obvious fat vacuoles, but these are visible in other parts of the parenchyma. The Mallory–Denk bodies consist of clumps and skeins of dense eosinophilic material, which sometimes forms a ring around the nucleus. When they are difficult to identify, positive immunostaining for p62 or ubiquitin is helpful[75,76] (**Fig. 7.19**). Swollen hepatocytes in steatohepatitis have reduced or absent filaments of keratins 8 and 18 (K8 and K18),[77] demonstrable by using specific K8 and K18 immunostains.[36,40] This feature can be used to distinguish steatohepatitic swelling from certain other causes of ballooning such as that seen in viral hepatitis where keratin 8/18 staining is preserved.[36] This can be demonstrated with specific immunostains.[36] Hepatocytes may also contain megamitochondria, which are rounded or elongated eosinophilic bodies from 2 to 10 μm across (**Fig. 7.20**). These can be distinguished from Mallory–Denk bodies by their more definite outline and by red staining with chromotrope–aniline blue (CAB); Mallory–Denk bodies usually stain blue with the latter. Megamitochondria can be found in both ASH and NASH as well as in the livers of alcohol abusers in the absence of steatohepatitis.[78–81] Crystalline intramitochondrial inclusions can be seen on transmission electron microscopy in the giant mitochondria of NASH.[81]

The inflammatory infiltrate is characteristically rich in neutrophils, but lymphocytes are also present. These are mainly T cells of CD4 and CD8 phenotype, and are found both in areas of steatohepatitis and in portal tracts.[82] Neutrophils surround or even infiltrate ballooned, Mallory–Denk body-containing hepatocytes (**Fig. 7.21**). Macrophages and other sinusoidal cells take part in the process. Kupffer cells may contain fat vacuoles.[82] Both macrophages and sinusoidal endothelial cells may contain stainable iron.[57]

Fibrosis is an integral part of the lesion of steatohepatitis. The most characteristic form of fibrosis is pericellular ('chicken-wire' fibrosis). Delicate or thicker strands of collagen surround ballooned hepatocytes to form a network which is well seen with trichrome stains (**Fig. 7.22**) and less easy to detect in reticulin preparations. The location corresponds to that of the cell damage and inflammation. In severe steatohepatitis, the fibrosis extends to the portal tracts as well as between perivenular areas, forming fibrous bridges often

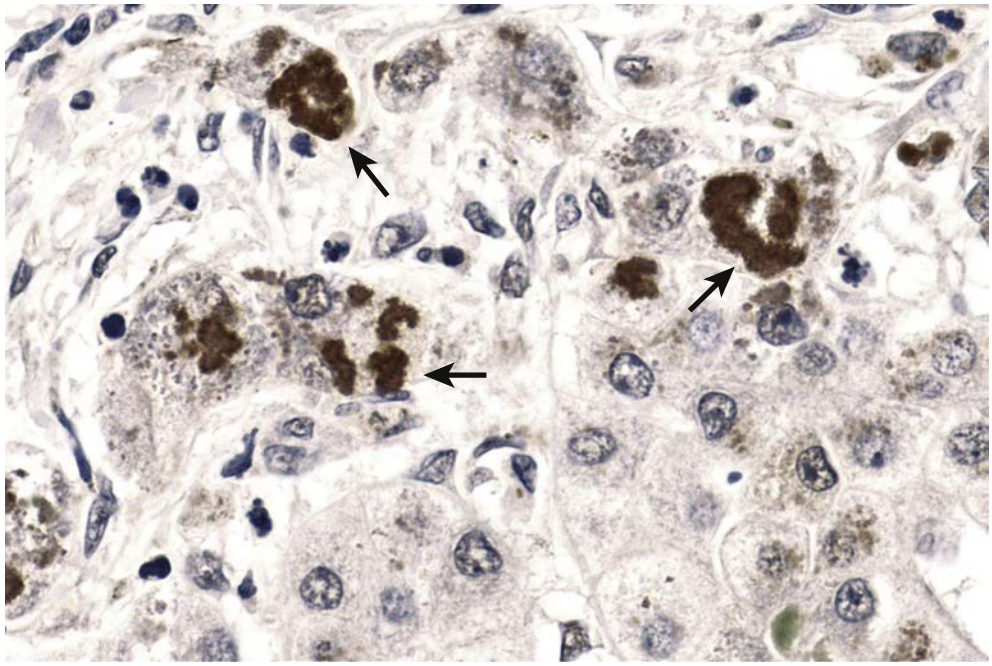

Fig. 7.19 Mallory–Denk bodies. The Mallory–Denk bodies in this example of steatohepatitis stain strongly for ubiquitin (arrows). (Needle biopsy, specific immunostain for ubiquitin.)

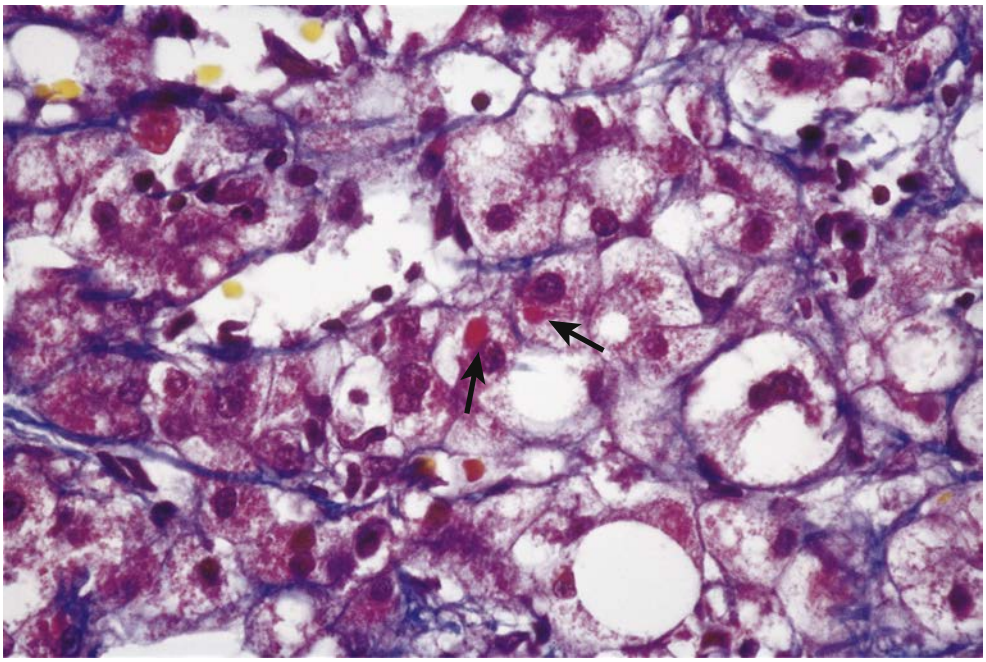

Fig. 7.20 Alcoholic steatohepatitis. Two bright red giant mitochondria are marked with arrows. Collagen fibres, stained blue, are seen around ballooned hepatocytes. (Needle biopsy, CAB.)

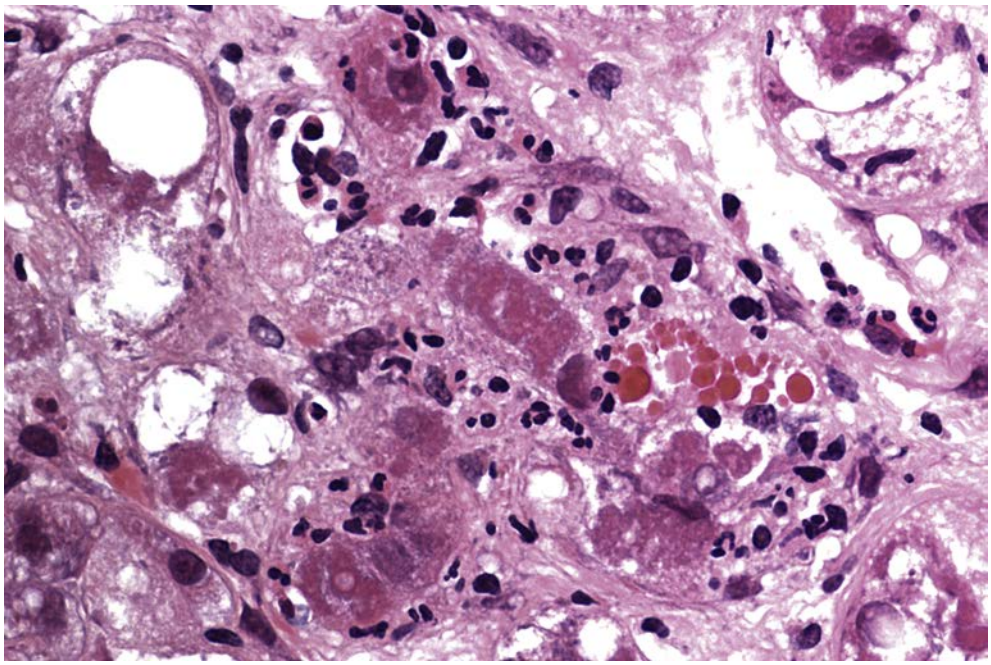

Fig. 7.21 Alcoholic steatohepatitis. Hepatocytes contain abundant Mallory–Denk bodies and neutrophils. (Needle biopsy, H&E.)

accompanied by a ductular reaction (**Fig. 7.23**). The evolution of such bridging fibrosis is similar to that seen in NASH.[83a,84a] Portal fibrosis is sometimes seen in the absence of the pericellular component.

These are the histological features of a classic, fully developed steatohepatitis. Like all pathological processes, however, steatohepatitis is an evolving lesion, which also varies in severity. For these reasons liver biopsy may show less obvious changes, not readily

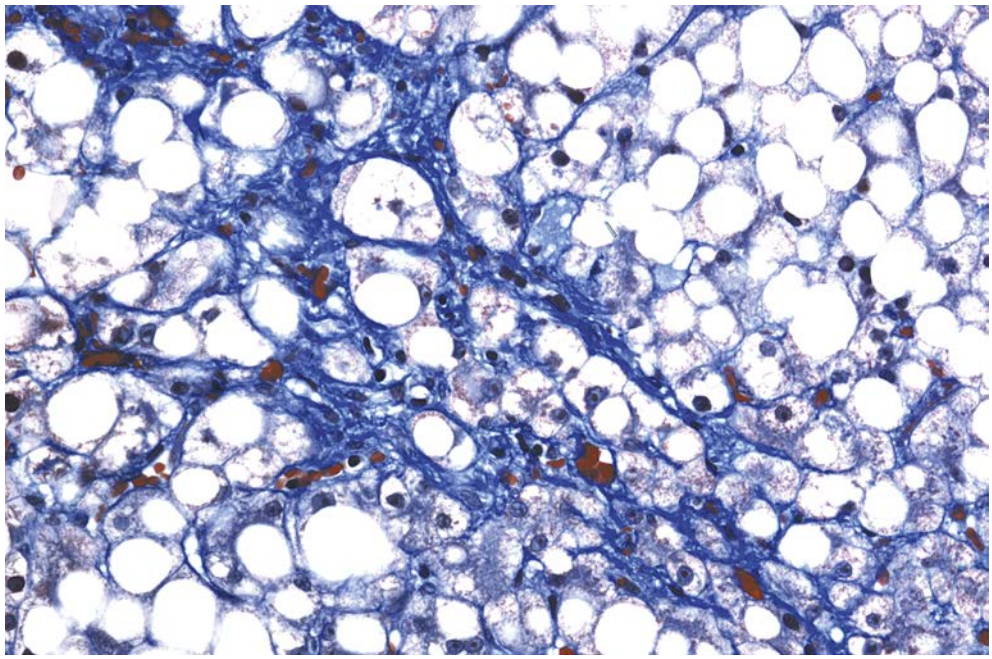

Fig. 7.22 Pericellular fibrosis. Collagen fibres, stained blue, form a meshwork in this example of steatohepatitis. (Needle biopsy, CAB.)

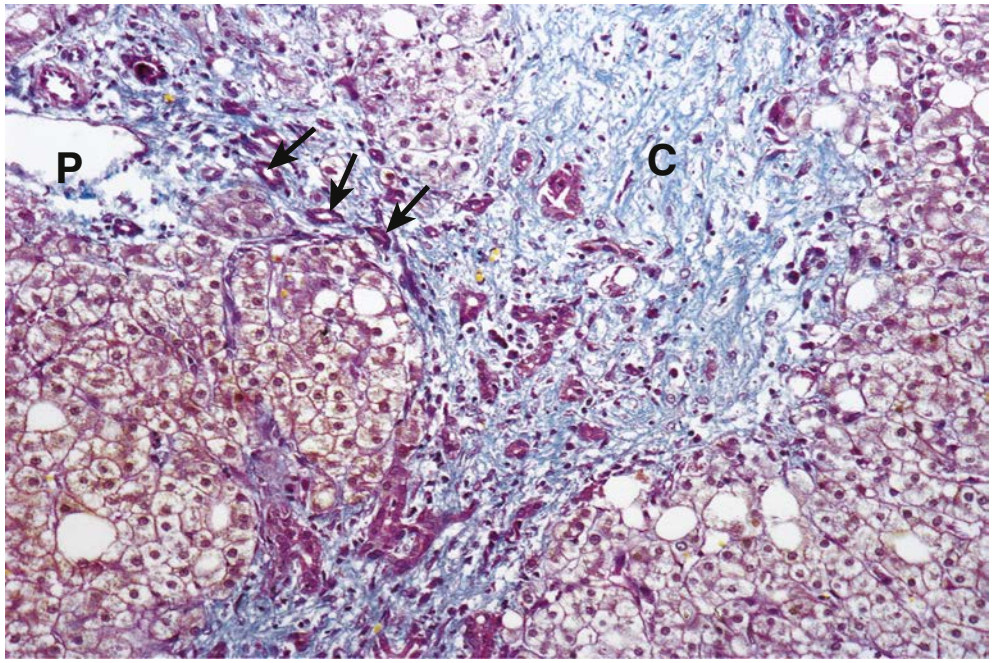

Fig. 7.23 Fibrosis in alcoholic steatohepatitis. Abundant collagen (C) has been laid down in a perivenular area, linked to a portal tract (P) by a fibrous bridge with ductular reaction (arrows). (Needle biopsy, Martius scarlet blue.)

recognised as part of the spectrum. Mallory–Denk bodies may be absent or not demonstrable in the biopsy sample. The inflammatory infiltrate may be predominantly lymphocytic, and pericellular fibrosis may be slight or undetectable. In a small minority of patients the only indication of a probable steatohepatitis is finding a few swollen, Mallory–Denk body–containing hepatocytes without associated inflammation. This should always be

reported and regarded as a sign that the patient may be at risk of progressive disease, which is more important from the point of view of patient management than the definition of minimal diagnostic criteria. In NASH, improvement in these histological features of steatohepatitis through weight loss and medications is associated with improvement or regression of fibrosis.[84b] The finding of pericellular fibrosis without any of the other changes of steatohepatitis may reflect past steatohepatitis.

Box 7.4 Main causes and associations of non-alcoholic steatohepatitis

Obesity
Diabetes mellitus
Metabolic syndrome
Hyperlipidaemia
Gastrointestinal surgery for obesity
Drugs and chemicals (e.g. amiodarone, tamoxifen, petrochemicals[79])

Non-alcoholic steatohepatitis

NASH has assumed increasing importance in recent years and the diagnosis is now a major clinical consideration when liver function tests are abnormal but viral markers are negative. The main clinical associations are listed in **Box 7.4**. It is important to note that while obesity, diabetes and the metabolic syndrome are common associations with NASH, patients are not all obese,[85] and hyperlipidaemia and other disorders of lipid metabolism may need to be investigated. Certain drugs (amiodarone,[86] calcium channel blockers,[87] tamoxifen[88]) and toxins[89] and rare disorders such as Weber–Christian disease[90] (nodular panniculitis) are other possible causes. NASH can affect men, women, the elderly[91,92] and also children.[50] In **paediatric NASH**, a histological picture of steatosis, portal fibrosis with or without fibrous septa and a mainly lymphocytic infiltrate (**Fig. 7.13**) is more common than the typical perivenular lesion of adult NASH; in the paediatric population this is referred to as NASH type 2.[50] Normal or mildly elevated serum alanine aminotransferase does not exclude the presence of significant steatosis or fibrosis in this group.[93]

There is extensive literature on the pathogenesis of NASH, some of it cited under 'General reading' at the end of this chapter. The exact mechanisms have not been fully elucidated, but many of the important factors involved have been defined. These include insulin resistance,[94] excess of free fatty acids in hepatocytes, lipid peroxidation[95] and oxidative stress.[96] Venous obstruction[97] and activation of Notch receptor protein signalling[98] may be important in the progression to cirrhosis. An element of genetic predisposition is likely,[99,100] and NASH has been reported in kindreds.[101] Some of the aforementioned factors, such as excess of free fatty acids and oxidative stress, are common to NASH and ASH and help to explain their similarity. It bears noting that although considerable basic science research on ASH and NASH has used animal models (chiefly mice), one of the key histological criteria of steatohepatitis, hepatocyte ballooning, has not to date been reproduced in murine models.[102]

The histological lesion in NASH (**Fig. 7.24**) is as described under the 'Pathological features of steatohepatitis' section, but may not be as severe as that in ASH. The presence of abundant neutrophils and Mallory–Denk bodies should therefore lead to a suspicion of alcohol abuse. Glycogen vacuolation of nuclei is common in NASH.[73a] Occasionally the lobular changes appear to be periportal rather than perivenular,[103] but this may reflect the difficulty of accurate localisation in two-dimensional sections. The pericellular fibrosis of NASH is very like that of ASH and is illustrated in **Fig. 8.9**. It is associated with hepatocellular injury, as shown by ballooning degeneration and Mallory body formation.[104] As described earlier, in some patients fibrosis is confined to the portal areas.[51,105] Hepatocytes damaged by pathogenetic factors active in NASH may show impaired regeneration (replicative senescence), and such affected liver cells may display positive nuclear staining for the cell cycle inhibitor p21 on immunostaining.[83a] In this setting, activation of periportal progenitor/stem cells to form a ductular reaction in tandem with fibrosis contributes to the bridging fibrosis and architectural obscuration seen in NASH as it progresses towards cirrhosis.[83a] In some cases, ductular reaction becomes prominent in centrilobular regions as fibrosis and stage progress.[106] Even in later stages of ASH and NASH, some portal tracts may be spared, and this can be a diagnostically helpful finding, further pointing to the

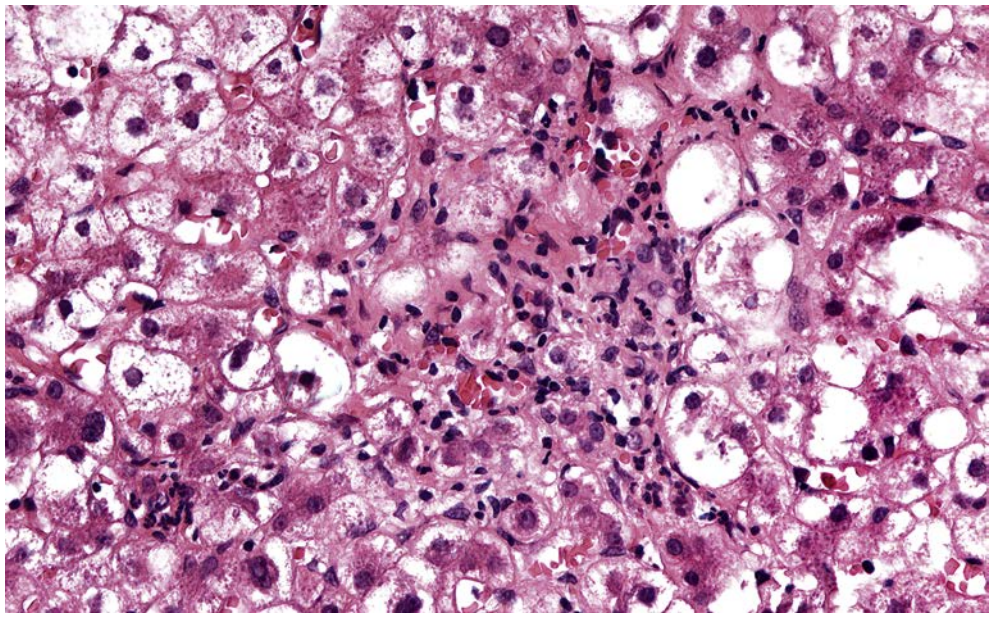

Fig. 7.24 Non-alcoholic steatohepatitis. There is steatosis, hepatocellular ballooning and infiltration by neutrophils, as in the alcoholic counterpart. (Needle biopsy, H&E.)

centrilobular regions as the site of the initiating insult. The presence of spared portal tracts at the centres of cirrhotic nodules surrounded by fibrous septa bridging between central veins may render an appearance of 'reversed lobulation' (**Ch. 10**).

Progression of the lesion to cirrhosis is variable but often slow.[107] Like other forms of cirrhosis, it carries the risk of liver failure and hepatocellular carcinoma.[108–110] Clinical features associated with NASH are also common in patients who have received diagnoses of 'cryptogenic cirrhosis', many of whom represent the late stage of NASH[111–114] in which little or no histological evidence of steatosis or steatohepatitis remains (**Fig. 7.25**). Loss of steatosis has been linked to decreased hepatic delivery of insulin and fatty acids due to portal hypertension as well as abnormal serum adiponectin levels.[115,116] Such 'burnt-out' cases of NASH sometimes show sufficient portal and septal chronic inflammation as to suggest the end stage of a chronic hepatitis, or, alternatively, show bland fibrosis surrounding regenerative nodules. The periphery of nodules near fibrous septa and portal tracts should be carefully examined for residual evidence of hepatocyte ballooning and/or Mallory–Denk bodies (**Fig. 7.25, inset**).

A scoring system for NASH has been devised, allowing semi-quantitative assessment and reporting of liver biopsy changes.[117–119] Separate scores are allotted for the severity of the hepatocyte damage and inflammation on the one hand, and for fibrosis and cirrhosis on the other (**Table 7.1**). As in the case of scoring in chronic viral hepatitis (**Ch. 9**), the resulting numbers must be regarded as categories rather than measurements.

For clinical and clinicopathological studies that require semi-quantitative data for liver pathology, the NAS score (NAFLD activity score) and SAF score (Steatosis-Activity-Fibrosis score) can be used (**Tables 7.2 and 7.3**). The NAS score evaluates the unweighted sum of steatosis (scored 0–3), lobular inflammation (scored 0–2) and hepatocellular ballooning (scored 0–2) in order to determine the presence of NASH (values ≥5 are considered NASH, and <3 'not NASH'). The SAF score, by contrast, also includes fibrosis.[120] Ultimately, the choice and use of a scoring system vary among pathologists and institutions, and the system(s) used may be selected for specific clinical and research needs. At minimum, though, the pathologist needs to be able to determine when steatohepatitis is present and what degree of fibrosis, if any, has developed, because these features have impact on therapy and prognosis.[83b,121a] Moreover, recent data have shown that of all the histological features

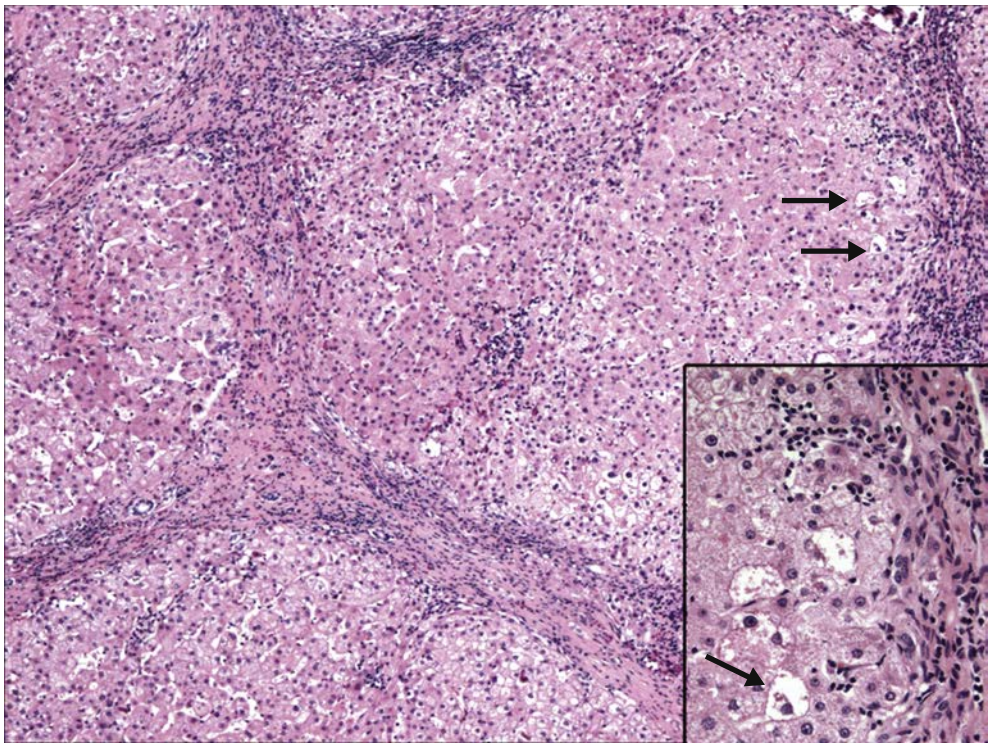

Fig. 7.25 Late non-alcoholic steatohepatitis (NASH) presenting as 'cryptogenic cirrhosis'. The non-descript cirrhotic nodules seen here are surrounded by fibrous septa with chronic inflammatory cells. There is focal periseptal interface hepatitis at upper right (arrows) where residual evidence of steatohepatitis is seen. Inset: Residual ballooning and intracellular Mallory–Denk bodies (arrow) are the remaining histological features of preceding non-alcoholic fatty liver disease and NASH. Note the absence of steatosis. (Explant liver, H&E.)

Table 7.1 A scoring system for steatohepatitis

Necroinflammatory grading	
Grade 1 (mild)	Steatosis (mainly macrovesicular) involving up to 66% of lobules; occasional ballooned perivenular hepatocytes; scattered neutrophils with or without lymphocytes; no or mild chronic portal inflammation
Grade 2 (moderate)	Steatosis of any degree; obvious ballooning (mainly perivenular); intralobular neutrophils, may be associated with perivenular pericellular fibrosis if evident; mild to moderate portal and intralobular chronic inflammation
Grade 3 (severe)	Panlobular steatosis; obvious perivenular ballooning and disarray; marked lobular inflammation; neutrophils may be concentrated in perivenular areas of ballooning and in areas of pericellular fibrosis if evident; portal inflammation mild or moderate
Fibrosis staging	
Stage 1	Pericellular fibrosis in perivenular areas, focal or extensive
Stage 2	As above, plus focal or extensive periportal fibrosis
Stage 3	Bridging fibrosis, focal or extensive
Stage 4	Cirrhosis

From Brunt EM, Janney CG, Di Bisceglie AM, et al. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol.* 1999;94:2467–2474.

Table 7.2 NAS Score (NAFLD Activity Score)

Component scores		
Steatosis grade	Lobular inflammation	Hepatocellular ballooning
0: <5%	0: None	0: None
1: 5%–33%	1: <2 foci/20× field	1: Mild, few
2: 34%–66%	2: 2–4 foci/20× field	2: Moderate/marked, many
3: >66%	3: >4 foci/20× field	
NAFLD Activity Score (NAS): 0–8		
Steatosis (0–3)	Lobular inflammation (0–3)	Ballooning (0–2)
Fibrosis*		
None 1a. Mild zone 3 sinusoidal fibrosis, requires trichrome stain to identify 1b. Moderate zone 3 sinusoidal fibrosis, may be appreciated on H&E 1c. Portal fibrosis only 2. Zone 3 sinusoidal fibrosis and periportal fibrosis 3. Bridging fibrosis 4. Cirrhosis		

NAFLD, non-alcoholic fatty liver disease. *Based on the use of Masson trichrome stain.
Data adapted from Kleiner et al.[63] with permission from Wiley Publishing.

Table 7.3 Steatosis-Activity-Fibrosis (SAF) histological scoring system for NAFLD

Steatosis grade (S): 0–3	Hepatocyte ballooning 0–2
S_0: <5%*	0: None
S_1: 5%–33%	1: Clusters of hepatocytes with rounded shape and pale and/or reticulated cytoplasm
S_2: 34%–66%	2: Same as score 1 with enlarged hepatocytes (more than two times normal size)
S_3: >66%	
Lobular inflammation 0–2	Activity grade (A): 0–4
0: None	Sum of scores for ballooning and lobular inflammation
1: ≤2 foci per 20× field	A_1 (A = 1): Mild activity
2: >2 foci per 20× field	A_2 (A = 2): Moderate activity
	A_3 A_4 (A > 2): Severe activity
Fibrosis stage (F)	SAF score
F_0: No significant fibrosis	$S_{0–3}$ $A_{0–4}$ $F_{0–4}$
F_1: 1a: Mild zone 3 sinusoidal fibrosis (SF) 1b: Moderate zone 3 SF 1c: Portal fibrosis only F_2: Zone 3 SF with periportal fibrosis F_3: Bridging fibrosis F_4: Cirrhosis	

*Percentage of hepatocytes containing large- and/or medium-sized intracytoplasmic lipid droplets.
NAFLD, non-alcoholic fatty liver disease.
Data adapted from Bedossa et al.[120] with permission from Wiley Publishing.

seen in NASH, it is the presence of fibrosis and its severity that is independently associated with the long-term overall mortality, liver transplantation and liver-related events.[121b]

Histological differential diagnosis

The histological differential diagnosis of steatohepatitis includes other forms of hepatitis. In viral hepatitis and AIH the infiltrating cells are lymphocytes and plasma cells rather than neutrophils. In acute viral hepatitis there is collapse of the reticulin framework but the 'chicken-wire' pattern of pericellular fibrosis is not seen. There may be steatosis in patients with chronic hepatitis C, particularly genotype 3 infection[122] (**Ch. 9**). Chronic hepatitis C accompanied by steatohepatitis is sometimes seen because of virus-related or co-morbid conditions associated with insulin resistance.[123] The fibrosis of venous outflow obstruction is usually linear and parasinusoidal rather than pericellular, but sometimes the hepatic fibrosis of long-standing cardiac disease can resemble that of steatohepatitis. The presence of congestion and absence of other features of steatohepatitis should make the diagnosis clear.

In chronic cholestasis with or without cirrhosis, hepatocytes near fibrous septa are typically ballooned and may contain Mallory–Denk bodies as well as bilirubin. Neutrophils are also seen. The correct diagnosis is made by attention to the location of the lesion, the general absence of steatosis, the presence of copper and copper-associated protein in the affected periportal hepatocytes, and to clinical circumstances. Amiodarone hepatotoxicity shows a similar periportal predilection of Mallory bodies and inflammation, often with little or no steatosis.[124] Ballooning and Mallory–Denk bodies are also features of Wilson's disease; again, confusion with steatohepatitis is unlikely.

Because steatohepatitis is common in some populations, it is quite often found together with the changes of another liver disease in the same biopsy. Documented diseases coexisting with steatohepatitis include chronic hepatitis, primary biliary cirrhosis, iron storage disorders, drug-induced liver injury and metabolic disorders.[125] The pathologist should therefore consider whether all the changes seen in a biopsy can be explained by steatohepatitis alone.

Other alcohol-related liver lesions

The pathological features of ASH have been described earlier. A wide variety of other changes may be found in liver biopsies from drinkers (**Box 7.5**). In some alcohol abusers the liver is histologically normal or shows only mild macrovesicular steatosis. Portal tracts may contain lymphocytic infiltrates in the absence of other features of hepatitis.[126]

Box 7.5 Liver lesions in the alcoholic

Steatosis
Macrovesicular
Microvesicular (foamy degeneration)
Steatohepatitis
Megamitochondria
Siderosis
Fibrosis
Pericellular
Perivenular
Portal
Cirrhosis
Hepatocellular carcinoma
Effects of non-hepatic alcohol-related diseases

Alcoholic foamy degeneration

Alcoholic foamy degeneration[127] is a relatively rare, potentially life-threatening condition characterised by extensive microvesicular steatosis in perivenular areas. Macrovesicular fat may be seen elsewhere. There may be cholestasis, fine fibrosis and scanty Mallory bodies, but inflammation is minimal or absent and the condition is thus distinct from ASH. Biochemical and histological features of cholestasis have been described, including some cases with exceedingly high serum aspartate aminotransferase and gamma glutamyl transferase levels compared to typical ASH.[128a,128b]

Fibrosis

Fibrosis is occasionally seen in drinkers in the absence of severe steatosis or steatohepatitis. Perivenular fibrosis may be

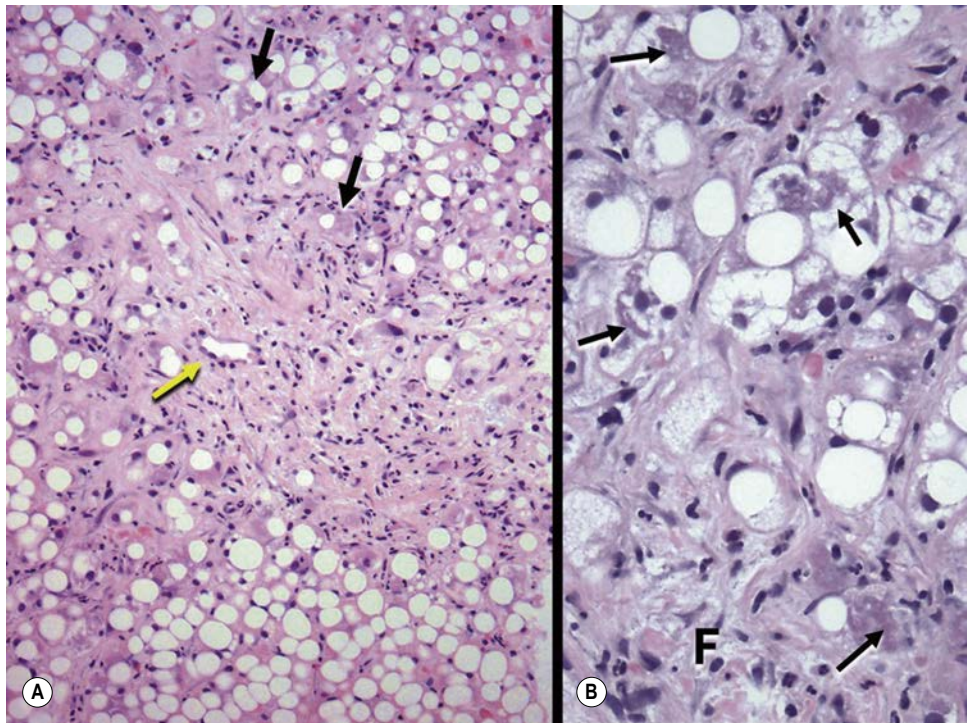

Fig. 7.26 Sclerosing hyaline necrosis. A: The efferent vein (yellow arrow) is surrounded by dense fibrosis and inflammation in this alcohol-related lesion. The adjacent perivenular hepatocytes are ballooned and contain Mallory-Denk bodies (black arrows). **B:** Higher magnification of the perivenular region showing fibrosis (F) with nearby ballooned hepatocytes, inflammation and numerous Mallory-Denk bodies (black arrows). (Needle biopsy, H&E).

found with or without steatosis or steatohepatitis. Dense perivenular scarring with nearby Mallory–Denk bodies and steatohepatitis is occasionally seen with heavy alcohol use (**sclerosing hyaline necrosis**)[129,130] (**Fig. 7.26**). Pericellular fibrosis is an important component of steatohepatitis, as already noted, and should always be looked for with the help of a collagen stain. When it is found in the absence of the other features of steatohepatitis, it may represent the remnant of a previous episode of this lesion. As such, it is a warning that the patient may be at risk of progressive disease if the cause is not removed. When the fibrosis is portal (**Fig. 7.27**), the possibility of associated biliary disease, alcoholic pancreatitis or coexisting viral hepatitis should be considered.

Fetal alcohol syndrome

In the fetal alcohol syndrome, children of mothers abusing alcohol during pregnancy have fatty livers and perisinusoidal and portal fibrosis.[131]

Cirrhosis

Cirrhosis in the alcoholic develops as a result of increasing fibrosis in steatohepatitis, together with nodular regeneration of the surviving parenchyma. There may also be other routes to cirrhosis, not involving steatohepatitis, but these are difficult to prove. Because steatohepatitis tends to involve all lobules, the cirrhosis is usually micronodular at first (**see Figs 10.12 and 10.13**). As the regeneration nodules enlarge, the cirrhosis remodels to a macronodular pattern and the original cause of the cirrhosis becomes more difficult or even impossible to establish on histological grounds. Venous occlusion is common,[132,133] and may be missed unless stains for collagen or elastic tissue are examined. Hepatocellular carcinoma may develop within the cirrhotic liver.

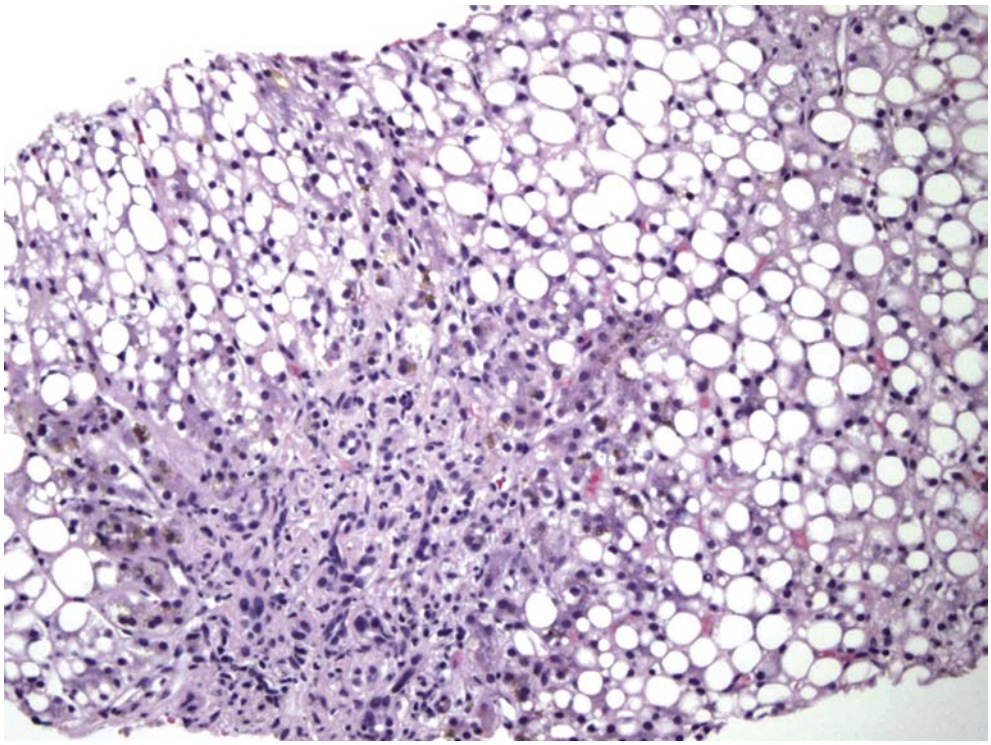

Fig. 7.27 Portal fibrosis in an alcoholic. A fibrotic portal tract with a stellate appearance is seen in this markedly fatty liver. Several bile ductular structures are evident within the fibrosis. The patient had a history of pancreatitis. (Needle biopsy, H&E.)

Other lesions

Alcohol-related lesions affecting organs other than the liver may cause liver changes. Chronic alcoholic pancreatitis has already been cited as a cause of portal fibrosis.[134] In patients with alcoholic cardiomyopathy the changes of right-sided heart failure may be found.

Finally, alcohol-related liver disease may coexist with other, non-alcohol-related liver diseases such as chronic hepatitis C. Alcohol consumption appears to accelerate the progression of fibrosis in hepatitis C.[135]

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CHAPTER

8 Drugs and Toxins

Introduction

This chapter deals with the pathology of the important liver lesions attributed to drugs and toxins, with their recognition and with their differential diagnosis. There are hundreds of hepatotoxic drugs and other chemicals,[1] and new reports of adverse drug reactions appear regularly in the literature under the acronym DILI (drug-induced liver injury). Heightened awareness of DILI during the last two decades has resulted in the creation of multicentre networks and databases in the United States, the United Kingdom, Europe and Asia which serve as ongoing resources for reporting and evaluation of new cases, data retrieval and correlation, phenotype characterisation and standardisation of nomenclature.[2] The LiverTox website (http://livertox.nih.gov/index.html), developed by the Liver Disease Research Branch of the National Institute of Diabetes and Digestive and Kidney Diseases and the National Library of Medicine in the United States, is a new and easily accessible source of information on over 1200 different medications, herbal agents and supplements. Other search engines available on the internet, such as PubMed, are additional resources to consult when DILI is suspected. If a liver biopsy is obtained in order to determine the cause of hepatitis, jaundice, acute liver failure or other type of liver disease, the pathologist should bear in mind that a drug cannot be exonerated simply because an adverse reaction has not been reported; there is always a first time.

Chemical injury is not confined to drugs listed in pharmacopoeias. Herbal medicines and dietary supplements,[3–11] illicit drugs,[12–20] criminally administered poisons,[21] industrial chemicals,[22–25] vitamins[26,27] and foods[28,29] have all been held responsible for liver disease. Drugs used for the treatment of liver disease have themselves been suspected of causing liver damage.[30]

In his foreword to the second edition of Stricker's *Drug-Induced Hepatic Injury*,[31] Zimmerman wrote: 'virtually all known acute and chronic hepatic lesions can result from drug injury'. This important observation implies that drugs should be considered as a possible cause of any liver lesion found on biopsy, but some lesions are more often produced by drugs than others. Hepatocellular necrosis, hepatitis and cholestasis in particular should arouse a greater degree of suspicion, especially if no other cause has been found. Also, some groups of drugs are associated with particular kinds of injury; non-steroidal anti-inflammatory drugs (NSAIDs), for example, are often associated with hepatocellular injury, while neuroleptic drugs mostly cause cholestasis. However, these are generalisations and a drug which causes a dose-related hepatocellular necrosis in one patient may cause non-dose-related hepatitis, cholestasis or granulomas in another.[32,33a]

The diagnostic pathologist should be aware of the potential of drugs and other substances to cause this wide variety of acute and chronic liver lesions and should know which lesions are most likely to be drug induced. He or she should be familiar with their likely course and

outcome, and the main points of similarity and difference from other, non-drug-related liver diseases. Finally, the pathologist should know where to look up the effects of individual drugs. The LiverTox site is found at http://livertox.nih.gov/php/searchchem.php.

Classification and mechanisms

Drugs may be regarded as producing liver injury in three main ways: *direct, indirect* and *idiosyncratic* hepatotoxicity[33b] (**Table 8.1**). **Direct (predictable) hepatotoxins** are those which predictably produce liver damage when taken in sufficient quantities: the chemical or its metabolites cause structural damage to cells and organelles. The type of damage is often characteristic of a particular drug; for example, the typical result of paracetamol (acetaminophen) overdose is hepatocellular necrosis and steatosis. Direct hepatotoxicity can often be studied in laboratory animals. This type of hepatotoxicity is also frequently **zonal** in distribution; examples of this are the perivenular lesions of paracetamol and carbon tetrachloride and the periportal necrosis seen in phosphorus and ferrous sulphate toxicity. In indirect hepatotoxicity the chemical interferes with a specific metabolic pathway or cell component. Agents in the class include monoclonal antibodies (e.g., checkpoint inhibitors and anti-tumor necrosis factor drugs such as infliximab) that may cause an immune-mediated hepatitis, sometimes with autoimmune histologic and serologic features.[33b,33c]

The more common kind of drug-related liver damage is **idiosyncratic (unpredictable)**. Only a small proportion of patients on a particular drug is affected, so that the adverse reaction is not detected in initial human trials. Antibiotics and psychoactive drugs are the most common cause of idiosyncratic DILI in Western countries.[31] Many different mechanisms for idiosyncratic hepatotoxicity have now been elucidated. They include individual genetic variation in the metabolism of drugs, and the development of immune reactions to a drug or its metabolites.[34] The immune reactions may be directed to neoantigens produced by the binding of reactive metabolites to hepatic drug-metabolising enzymes of the P450 system.[35,36] In some instances the distinction between an idiosyncratic and intrinsic drug reaction is difficult to make. Typical idiosyncratic damage may follow a small dose of the offending drug, and cannot easily be studied in the laboratory. With the exception of a few drugs shown to cause liver damage in patients using a particular metabolic pathway, idiosyncratic drug injury is

Table 8.1 Examples of liver lesions due to drugs and toxins.

Lesion	Example of substance
Intrinsic hepatotoxicity	
Microvesicular steatosis	Valproate
Phospholipidosis	Amiodarone
Hepatocellular necrosis	Paracetamol (acetaminophen)
Fibrosis	Vitamin A
Cholestasis	Contraceptive steroids
Venous occlusion	Pyrrolizidine alkaloids
Angiosarcoma	Vinyl chloride
Idiosyncratic hepatotoxicity	
Hepatitis	Isoniazid
Cholestasis	Amoxicillin–clavulanic acid
Granuloma formation	Allopurinol

unpredictable in the sense that the susceptibility of individual patients cannot be tested before the drug is given.

Most intrinsic hepatotoxins produce liver damage within a few hours or days, whereas in the idiosyncratic type of injury there is often a latent period of many days, weeks or months[37] before liver disease becomes apparent. The latent period tends to shorten with repeated administration of the drug. Because of the latent period and the tendency for idiosyncratic injury to mimic non-drug-related liver diseases, clinicians and pathologists need to be alert to the possibility of idiosyncratic drug injury if diagnostic errors are to be avoided. The clinician may be helped by compiling specific data[38] and by using a causality scale.[39,40] The pathologist may be helped by finding a suspicious or characteristic pattern of injury.[41] Conclusive proof that a particular drug or combination of drugs is responsible is often impossible to obtain, although rechallenge (usually inadvertent) can provide strong circumstantial evidence. Liver injury may follow inadvertent rechallenge many years after a first episode.[42] Biochemical evidence of improvement after drug withdrawal is occasionally supported by a return to normal histology.[37]

Commonly implicated and newer agents

The growth of international databases and registries of drug hepatotoxicity has provided more comprehensive information pertaining to the risks and likelihood of specific drugs and other agents causing liver injury. For example, amoxicillin–clavulanate is the most implicated agent in DILI in prospective studies from Spain, the United States and Iceland.[43a,43b] Isoniazid and nitrofurantoin are also among the top five implicated drugs.

While such commonly implicated agents naturally warrant consideration in cases of suspected DILI, new and newly popularised agents also require exclusion. The immune checkpoint inhibitors are a case in point for which the histologic features of liver injury have become better recognised as their use in cancer immunotherapy has grown in recent years.[44–49] Immune checkpoint inhibitors block two downstream regulators of immunity: cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death ligand 1 (PD-L1). Ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-L1) are commonly used checkpoint inhibitors that have resulted in fibrin-ring granulomas[45] and lobular hepatitis[46] on liver biopsy. Pembrolizumab also has been associated with prominent lobular hepatitis, mild bile-duct injury with mild portal vein endotheliitis and numerous necroinflammatory collections of Kupffer cells and lymphocytes resembling microgranulomas.[47] Certain checkpoint inhibitors also may cause biliary injury, such as pembrolizumab-associated secondary sclerosing cholangitis.[49] Even the highly successful direct-acting antiviral agents such as sofosbuvir that have revolutionised the treatment of hepatitis C virus are not necessarily exempt from hepatotoxicity, as a recent study has suggested.[50] Biologic agents used in the treatment of rheumatic diseases require careful monitoring because serious liver and systemic injury may occur, such as the development of hepatosplenic T-cell lymphoma following the use of tumour necrosis factor-alpha (TNF-α) inhibitors infliximab, adalimumab and etanercept.[51]

Morphological categories

The categories described in the following sections represent the main changes attributed to drugs and toxins, apart from alcohol-related liver damage (**Ch. 7**), neoplasms (**Ch. 11**) and vascular lesions (**Ch. 12**). A mixture of lesions may be found in the same liver: amiodarone, for example, produces both phospholipidosis and steatohepatitis, but by different mechanisms.[52] As already indicated, a single drug may give rise to different forms of hepatotoxicity in different patients. Phenylbutazone, for example, can cause necrosis, cholestasis, granuloma formation or combinations of these,[53] while the NSAIDs nimesulide and diclofenac can cause either severe hepatitis or cholestasis.[54,55]

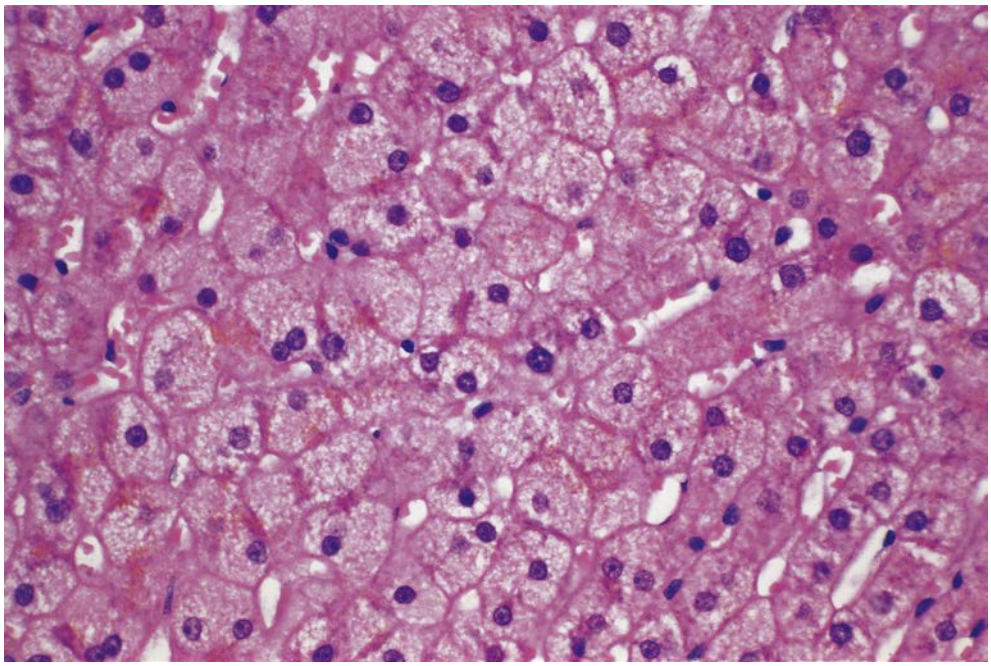

Fig. 8.1 Adaptation. Hepatocytes in this biopsy from a patient on antiepileptic drugs are enlarged and have abundant pale-staining cytoplasm. (Needle biopsy, H&E.)

Adaptation

Not all changes seen under the microscope necessarily represent liver damage. The increase in endoplasmic reticulum produced by long-term treatment with anticonvulsant drugs is commonly regarded as an adaptive phenomenon.[56,57] By light microscopy, this increase is seen as an abundance of pale-staining cytoplasm in hepatocytes (Fig. 8.1 and **see Fig. 4.4**), which is difficult to distinguish from simple abundance of glycogen on a haematoxylin–eosin (H&E)-stained section.

Non-hepatitic liver-cell damage

One of the most common manifestations of intrinsic hepatotoxicity is **steatosis**. As discussed in **Chapter 7**, this may be macrovesicular or microvesicular. Macrovesicular steatosis, in which the nucleus of the hepatocyte is displaced by one or more fat vacuoles easily visible by light microscopy, is produced by chlorinated hydrocarbons and methotrexate, for example. It is common in patients on total parenteral nutrition,[58,59] although underlying disease may also contribute to the liver changes.[60] In patients treated with gold compounds for rheumatoid arthritis, intralobular lipogranulomas (focal accumulations of lipid-containing macrophages) have been found to contain gold pigment in the form of fine black or brown granules. These were also seen within portal lipid droplets.[61]

Causes of the more serious microvesicular steatosis[62] (**Fig. 8.2**) include treatment with the anticonvulsant drug valproate[63] and with the nucleoside analogue fialuridine.[64] An increased risk of acute liver failure with valproate use is seen in individuals with underlying mutations in the *POLG1* gene for mitochondrial DNA polymerase gamma.[65] This leads to the combination of microvesicular steatosis with mitochondrial abnormalities, found also in Reye's syndrome (**Ch. 13**). Similar changes are reported after using zidovudine,[66] didanosine (**Fig. 8.3**) and other nucleoside reverse transcriptase inhibitors in highly active antiretroviral therapy (HAART) for acquired immunodeficiency syndrome (AIDS).[67–70] In the microvesicular form

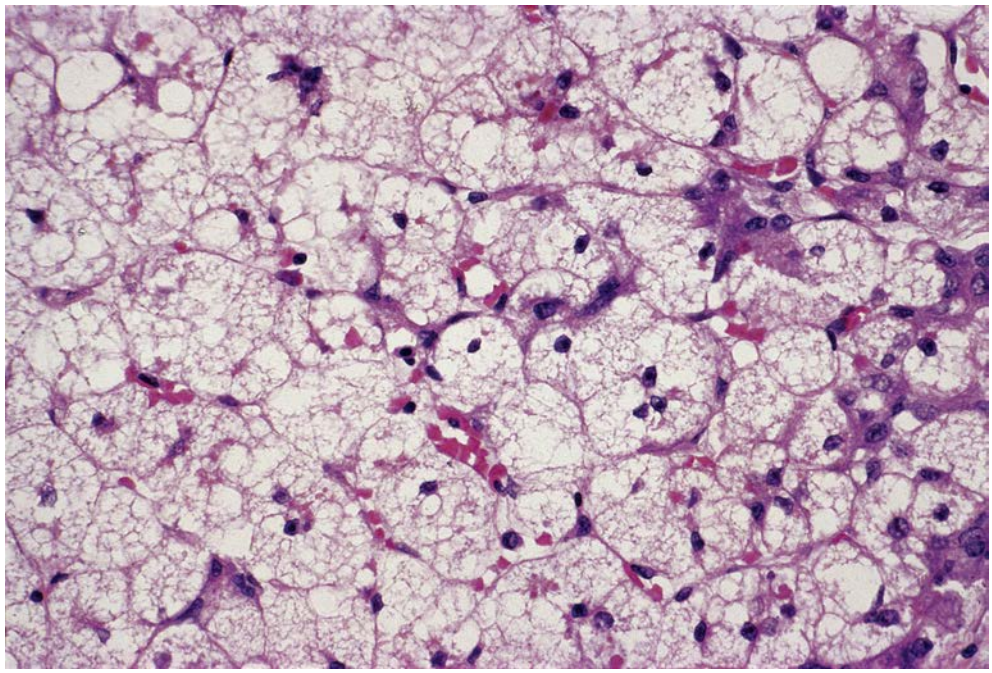

Fig. 8.2 Microvesicular steatosis. In this example of valproate toxicity, the hepatocytes are swollen and finely vacuolated. (Recipient liver at transplantation, H&E.) (The section was kindly provided by Professor BC Portmann, London, UK.)

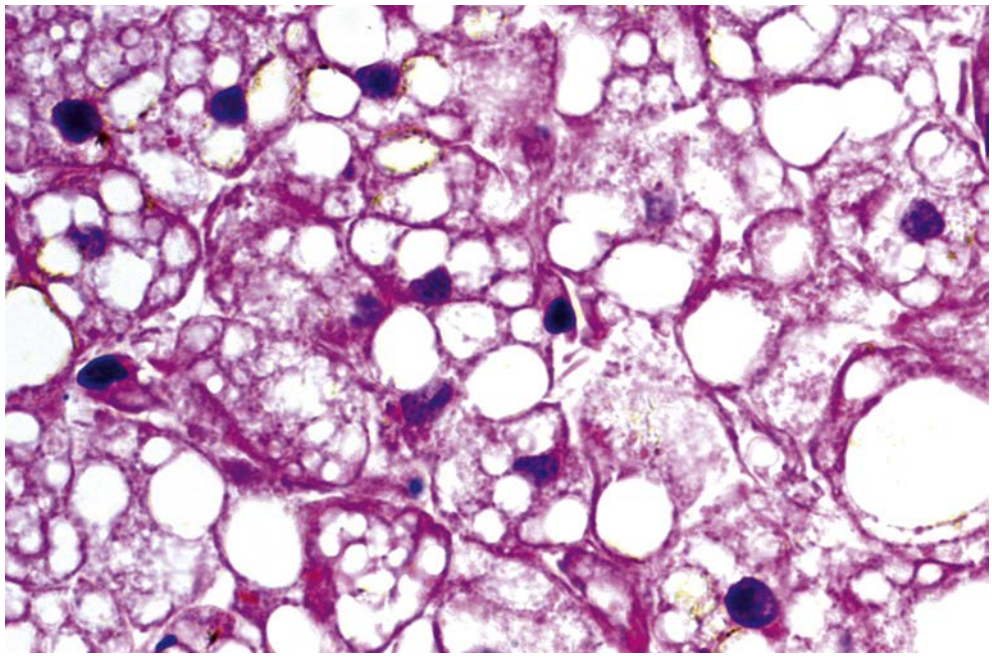

Fig. 8.3 Didanosine-induced microvesicular steatosis. Small-droplet fat vacuoles are prominent in hepatocytes, most of which show nuclei maintained in a central position. (Needle biopsy, H&E.)

of steatosis the fat within the hepatocytes is finely divided and is not always obvious with conventional stains. The hepatocyte nuclei remain in their normal central location, in contrast to macrovesicular steatosis. There is a variable degree of associated hepatocellular necrosis.

Several drugs, among them amiodarone[52] and trimethoprim-sulfamethoxazole (co-trimoxazole),[71] are causes of acquired **phospholipidosis**. Similar changes have been reported in patients receiving total parenteral nutrition.[72] Lamellar inclusions are seen within hepatocytes

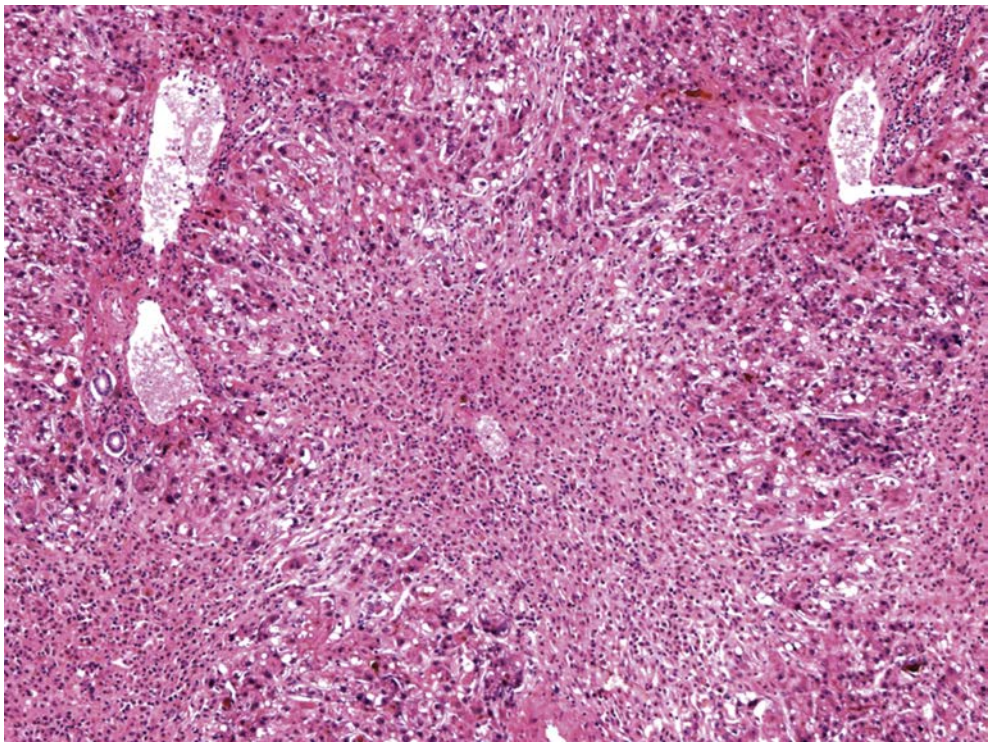

Fig. 8.4 Hepatocellular necrosis due to paracetamol (acetaminophen). Confluent necrosis with little inflammation is seen in a perivenular area. The surviving parenchyma near the portal tracts (upper left and upper right) shows mild steatosis and cholestasis. (Explant liver, N, H&E.)

and other cells by electron microscopy (**see Fig. 17.3**). Light microscopy of conventionally stained sections is not diagnostic.

In acute arsenic intoxication, a striking increase in hepatocyte mitoses has been reported, accompanied by ballooning, cholestasis and mild inflammation.[21] Markers of cell proliferation were also markedly increased.

An unusual form of cell injury is produced by cyanamide, used in alcohol aversion therapy.[73–75] Periportal hepatocytes contain large, pale-staining **cytoplasmic inclusion bodies**, giving the cells a superficial resemblance to the ground-glass cells of chronic type B hepatitis (**see Figs 4.4 and 9.13**). The inclusions are, however, orcein-negative and diastase–periodic acid–Schiff-positive.

Hepatocellular necrosis

Hepatocellular necrosis without the diffuse inflammatory lesion of hepatitis is usually a consequence of the intrinsic type of hepatotoxicity. A common example is suicidal or accidental overdose with the analgesic paracetamol.[76] Jaundice develops after an interval of days, during which available glutathione, which reacts with a toxic metabolite, is used up. The necrosis—like that of shock or heatstroke (**see Fig. 12.2**)—is most severe in perivenular regions (acinar zones 3) and is accompanied by little or no inflammation (**Fig. 8.4**). Kupffer cells contain brown ceroid pigment. Portal tracts usually remain normal. A few neutrophils and lymphocytes are sometimes also seen in necrotic regions, due to activation of innate immunity by damage-associated molecular pattern (DAMP) molecules such as high-mobility group box-1 (HMGB1) and keratin 18 released from necrotic hepatocytes.[77–79] Complete recovery is possible. While most paracetamol-induced necrosis follows suicidal overdose, it is occasionally found in habitual drinkers taking large doses in the high therapeutic range.[80]

Hepatocellular necrosis, sometimes accompanied by steatosis, is also a feature of cocaine intoxication,[12,14,16] glue sniffing and solvent abuse.[17,81] In most instances the necrosis is

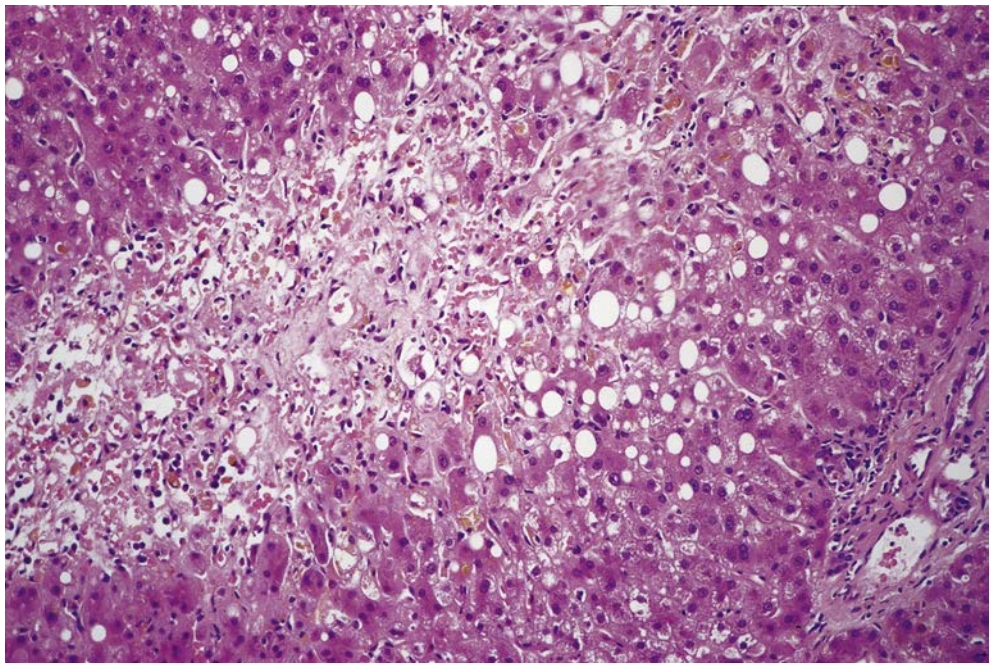

Fig. 8.5 Drug-induced liver injury: hepatitic type. In this acute hepatitis attributed to indometacin, necrosis in acinar zone 3 is well demarcated from the remaining parenchyma. The latter shows steatosis. Note the very mild portal inflammation (bottom right). (Needle biopsy, H&E.)

perivenular and mid-zonal (in acinar zones 3 and 2), but periportal (zone 1) necrosis has been reported in a cocaine user.[82] 'Ecstasy' (3,4-methylenedioxymethamphetamine [MDMA]) can cause a hepatitic lesion of the kind described in the next section,[18,19,83] but there may also be confluent hepatocellular necrosis as a result of concurrent hyperthermia.[20] Other agents capable of causing confluent necrosis include industrial hydrochlorofluorocarbons.[22]

Acute drug-induced hepatitis

A large number of drugs of different chemical structure and with widely differing pharmacological actions occasionally give rise to acute hepatitis, and any drug should be regarded as a potential offender. Acute drug-induced hepatotoxicity is of the idiosyncratic type. The histological lesion is very like that of acute viral hepatitis, and often indistinguishable from it (**Figs 8.5 and 8.6**). Incriminated substances include antituberculous drugs,[84] NSAIDs, anaesthetics,[85,86] herbal remedies[4] and many others.

In the idiosyncratic injury of hepatitic type the latent period between exposure to the drug and clinically evident liver disease ranges from a few days to several months or longer, with a long latent period sometimes making diagnosis difficult. However, correct diagnosis of idiosyncratic drug-induced hepatitis is most important, because inadvertent rechallenge may have serious consequences.

The hepatitis ranges in severity from a mild inflammatory lesion, sometimes combined with a cholestatic reaction (see the discussion under the 'Steroid-induced cholestasis' section), to severe and even fatal disease.[87] In milder cases, removal of the drug usually leads to rapid improvement. Later uncommon outcomes also include cirrhosis and the development of autoimmune hepatitis (AIH).[88]

Differential diagnosis

The possibility of drug idiosyncrasy should be considered in all patients with acute hepatitis, because in many cases the histological appearances are identical to those of viral hepatitis.

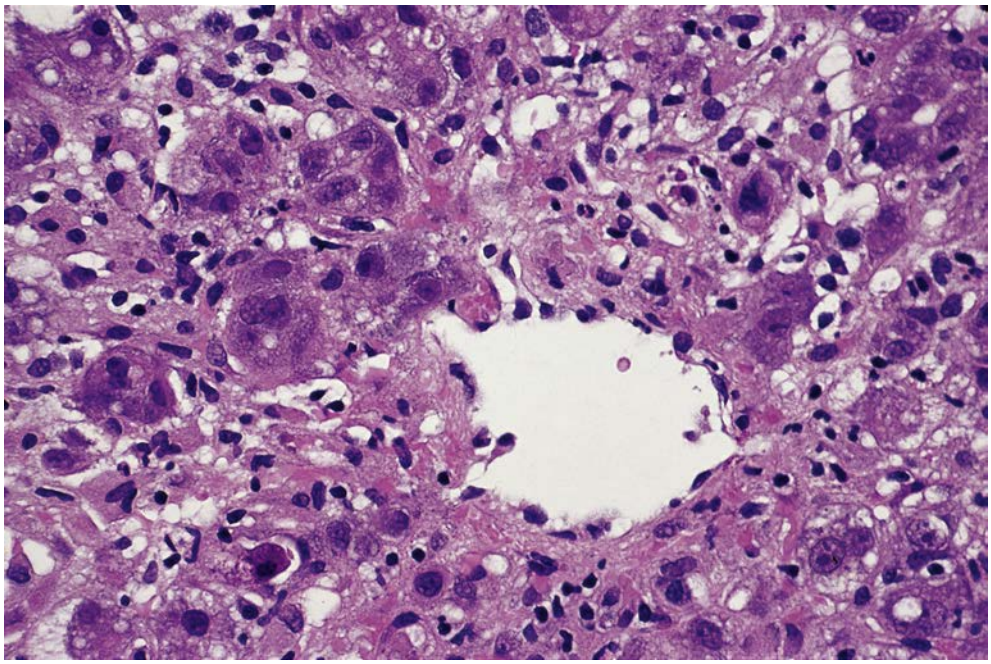

Fig. 8.6 Drug-induced liver injury: hepatitic type. There is a severe lobular hepatitis with disruption of liver-cell plates and apoptosis, attributed to ecstasy (3,4-methylenedioxymethamphetamine). The patient is the second of the two reported by Fidler and colleagues.[19] (Needle biopsy, H&E.)

Serological exclusion of hepatitis A, B and C infection is warranted and acute hepatitis E may also require consideration (see Chronic drug-induced hepatitis, below). A higher than usual degree of suspicion of drug hepatotoxicity should be aroused when the hepatitis is histologically unusual (**Box 8.1**). Well-demarcated centrilobular confluent necrosis (**Fig. 8.5**) is common. There may be a very mild lobular hepatitis together with canalicular cholestasis. The portal inflammatory reaction may be poorly developed or even absent. Conversely, the portal infiltrate may be unusually rich in neutrophils or eosinophils, although the latter are neither proof of drug aetiology nor necessary for its diagnosis. The presence of epithelioid-cell granulomas increases the likelihood that drug idiosyncrasy is the correct diagnosis.

Box 8.1 Features sometimes associated with drug-induced hepatitis

Demarcated perivenular (acinar zone 3) necrosis
Minimal hepatitis with canalicular cholestasis
Poorly developed portal inflammatory reaction
Abundant neutrophils
Abundant eosinophils
Epithelioid-cell granulomas

Chronic drug-induced hepatitis

Evolution of drug hepatotoxicity to chronic liver disease is relatively uncommon and usually requires prolonged or repeated exposure to the injurious agent.[89–91] The spectrum of histological changes includes chronic hepatitis, intrahepatic bile-duct injury, ductopenia and chronic cholestasis, fibrosis and/or cirrhosis[41] A condition closely resembling AIH, with positive serum autoantibodies and active lymphoplasmacytic interface hepatitis on biopsy, may develop with certain drugs[92,93] (in the United States, most often due to nitrofurantoin[92,94] and minocycline[92,93,95]). Other drugs, herbal products or dietary supplements have also been implicated, including methyldopa and statins (**Figs 8.7 and 8.8**) and other agents[93,96–99] (**Box 8.2**). The histological distinction between idiopathic AIH and DILI is often difficult.[100] Idiopathic AIH is favoured by the presence of more active interface hepatitis, portal, periportal and lobular plasma cells, rosettes, a higher stage of fibrosis and the presence of cirrhosis. Eosinophils may be seen in both AIH and DILI and

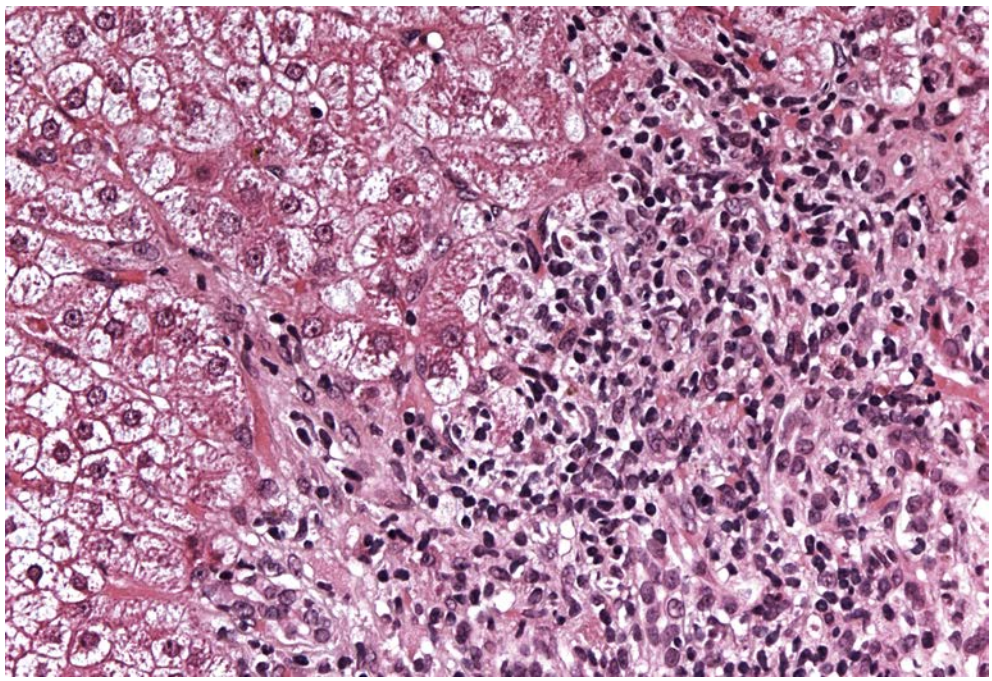

Fig. 8.7 Drug-induced chronic hepatitis. Liver damage, here attributed to methyldopa, has taken the form of extensive interface hepatitis. There is a heavy lymphoplasmacytic infiltrate. (Needle biopsy, H&E.)

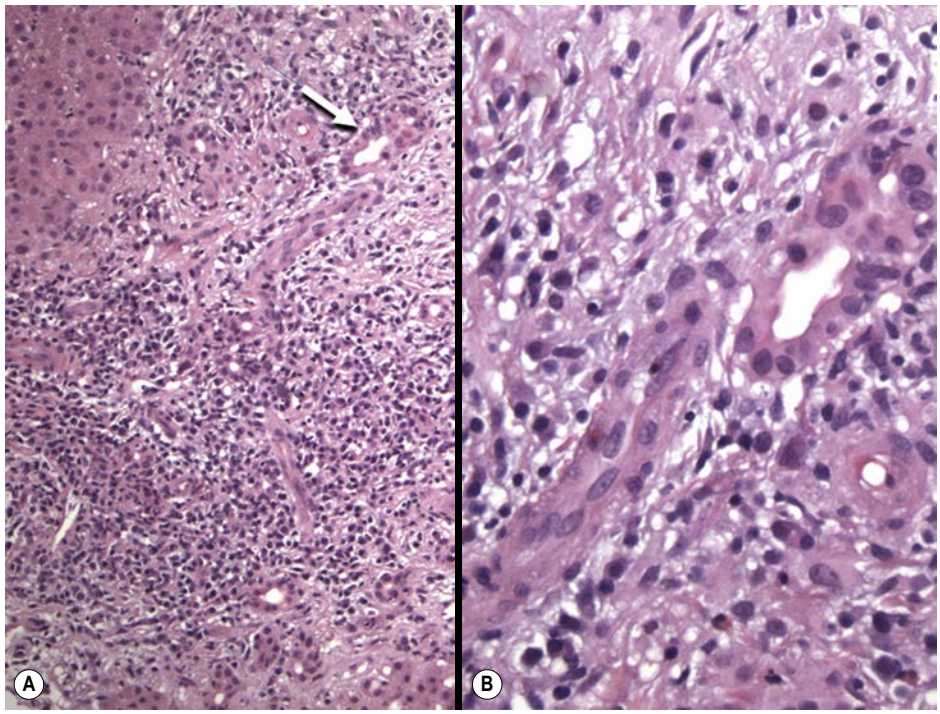

Fig. 8.8 Drug-induced liver injury-autoimmune hepatitis (DILI-AIH) associated with statins. The patient had recently begun a course of statin therapy and developed a robust hepatitis resembling autoimmune hepatitis. **A:** The portal tract shows diffuse and irregular expansion by lymphocytes and numerous plasma cells, with interface hepatitis and bile-duct injury (white arrow). **B:** Intraepithelial lymphocytes, duct cell vacuolisation, altered nuclear polarity and epithelial stratification are features of the bile-duct injury. After a course of steroids and withdrawal of the statin, 1 year later the patient's liver disease recurred and was determined to be autoimmune hepatitis, originally triggered by a statin. (Needle biopsy, H&E.)

therefore are not useful for discrimination.[100] By contrast, the presence of neutrophils in portal tracts and cholestasis within hepatocytes and bile canaliculi lend support for DILI.[100] Those cases where a drug has unmasked an underlying AIH often become clear when relapse occurs after withdrawal of immunosuppression.[98,101a]

Differential diagnosis

Chronic viral hepatitis and AIH constitute the major histological differential diagnosis of chronic drug-induced hepatitis. Because some cases of acute hepatitis A virus (HAV) infection show abundant portal and periportal plasma cells with interface hepatitis on biopsy (therein resembling idiopathic AIH), acute HAV infection should be excluded by serum testing for immunoglobulin M (IgM) antibody to HAV. Hepatitis E virus infection also should be considered in the differential diagnosis, particularly in recipients of organ transplants or other immunosuppressed individuals who may have become infected with indigenous, zoonotic HEV such as genotype 3.[101b]

Box 8.2 Agents associated with features of autoimmune hepatitis

Nitrofurantoin
Minocycline
Alpha-methyldopa
HMB-CoA (Hydroxy Beta-Methylbutyryl-Coenzyme A) reductase inhibitors (statins)
Hydralazine
Diclofenac (non-steroidal anti-inflammatory drug)
Tumour necrosis factor-alpha (TNF-α) antagonists (e.g. infliximab, adalimumab)
Interferons
Black cohosh (herbal)
Ma huang (herbal)

Steatohepatitis

Steatohepatitis refers to a specific form of hepatic injury characterised by steatosis, hepatocellular ballooning, Mallory body formation, inflammation and pericellular fibrosis, sometimes progressing to cirrhosis. The most common cause is alcohol abuse (Ch. 7). Drugs are among the causes of non-alcoholic steatohepatitis (NASH). Incriminated agents include synthetic oestrogens,[102] amiodarone[52,103] and tamoxifen[104–106] (Fig. 8.9). Similar changes are sometimes seen in patients on parenteral nutrition[58] and in industrial workers exposed to volatile petrochemical

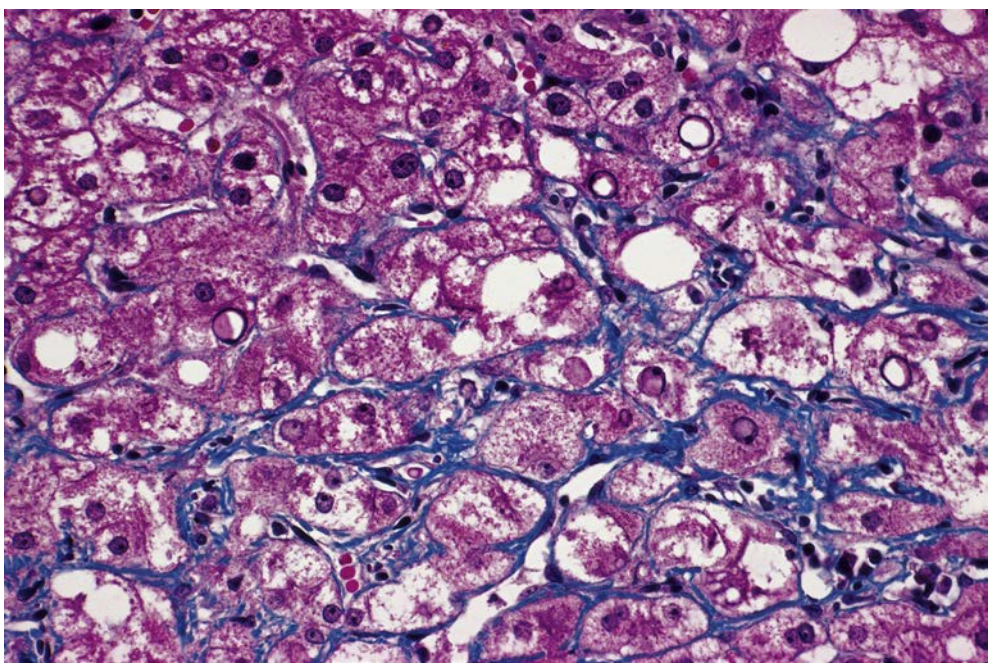

Fig. 8.9 Steatohepatitis attributed to tamoxifen toxicity. Hepatocytes in the lower part of the field are swollen and surrounded by collagen, stained blue. There is also steatosis and nuclear vacuolation. (Needle biopsy, trichrome.) The patient is the third of the three reported by Pinto and colleagues.[106] (The biopsy was kindly provided by Professor Amelia Baptista, Lisbon, Portugal.)

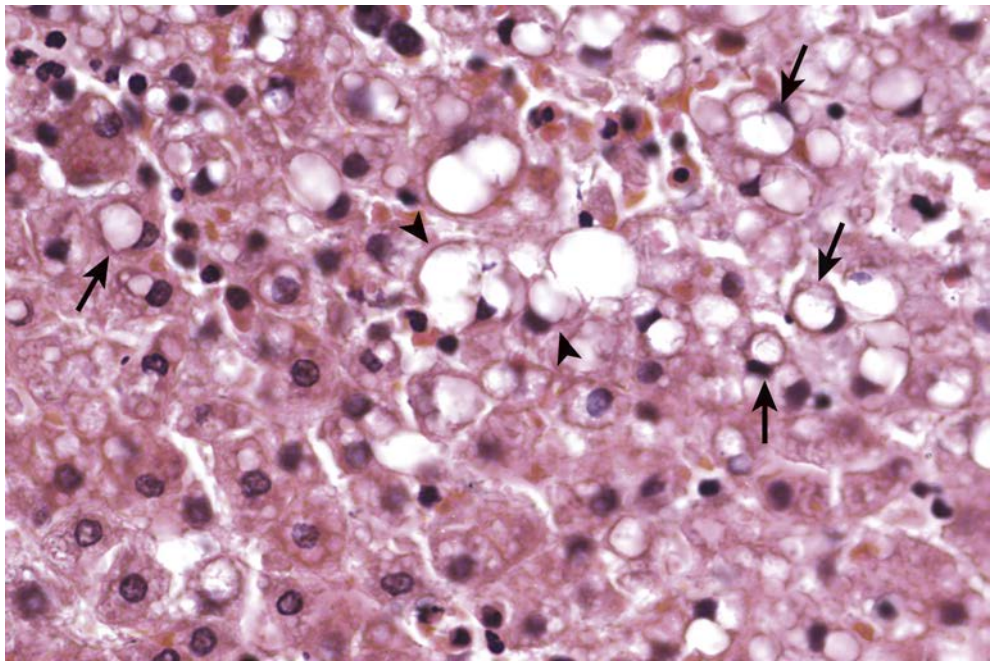

Fig. 8.10 Hypervitaminosis A. Prominent, hypertrophied stellate cells with lipid vacuoles and peripheral dark, compressed nuclei are seen between hepatocytes, in perisinusoidal spaces (arrows). Some of the stellate cells are multivesicular (arrowheads). (Needle biopsy, H&E.)

products.[23] In the case of amiodarone, steatosis itself may be mild or absent,[107] but otherwise there is a close resemblance to other forms of NASH and to alcoholic steatohepatitis, including the potential for cirrhosis. However, amiodarone-related steatohepatitis has a periportal predilection, in contrast to the perivenular distribution seen with other causes of steatohepatitis. It is interesting to note that some patients with reported drug-related steatohepatitis were also obese,[106] which raises the possibility of an interaction between drug and other factors.

Fibrosis and cirrhosis

As already stated, cirrhosis may result from chronic drug-induced hepatitis. Progressive fibrosis and portal hypertension in a non-hepatitic setting are known complications of long-term exposure to arsenic or vinyl chloride. Excess intake of vitamin A (hypervitaminosis A) affects hepatic stellate cells which may appear unusually prominent[108] (**Fig. 8.10**). Perisinusoidal fibrosis, veno-occlusive disease and cirrhosis[27] are other consequences.

Pathologists are sometimes asked to report on liver biopsies from patients given or about to receive long-term methotrexate for psoriasis or rheumatoid arthritis. Although methotrexate was initially considered to be a potent hepatotoxin, doubt has more recently been thrown on its potential to cause serious liver disease in the absence of additional risk factors.[109] These include regular or heavy alcohol intake[110] and obesity.[111] Significant liver injury is reputedly less common in patients with rheumatoid arthritis than in those with psoriasis. Histological abnormalities attributed to methotrexate include steatosis, hepatocyte pleomorphism, portal fibrosis and inflammation, formation of fibrous septa extending from the portal tracts (**Fig. 8.11**) and cirrhosis. A grading system for methotrexate liver injury was developed by Roenigk and colleagues which scores the degree of fat, inflammation and fibrosis.[112] Minor changes such as focal necrosis and steatosis are common in baseline pretreatment biopsies, and are presumably related to the underlying disease (e.g. psoriasis) or to additional risk factors. Periportal septum formation is more likely to be due to methotrexate, whereas fibrosis mainly in perivenular regions should lead to suspicion of alcohol abuse or NASH.

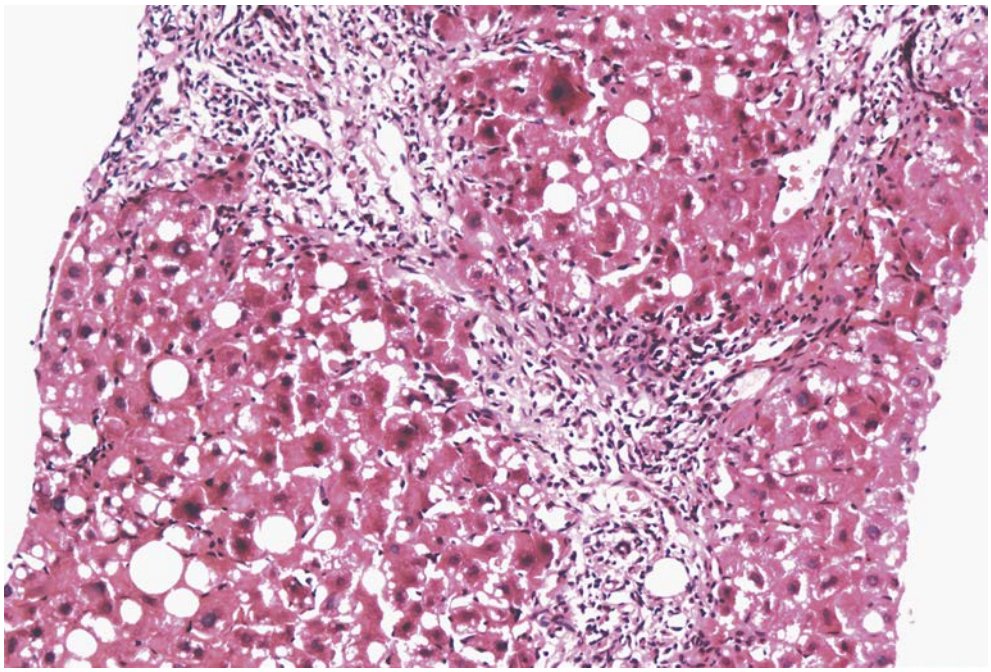

Fig. 8.11 Liver damage attributed to methotrexate. Two portal tracts in this field show chronic inflammation and fibrosis extending outwards. The parenchyma shows steatosis. (Needle biopsy, H&E.)

Steroid-induced cholestasis

Steroid-induced cholestasis[113,114] lies on the borderline between intrinsic and idiosyncratic hepatotoxicity. On the one hand, it is reproducible in laboratory animals, and some steroids cause biochemical abnormalities in humans in a predictable and dose-dependent manner. On the other hand, clinical liver disease cannot be predicted in the individual patient and is seen in only a small proportion of patients receiving anabolic or contraceptive steroids. Patients susceptible to contraceptive steroid-induced jaundice are also prone to developing cholestasis in late pregnancy.

The histological picture is one of canalicular cholestasis in perivenular areas, with little or no necrosis or inflammation beyond that attributable to the cholestasis itself (**Fig. 8.12**). Isolated hepatocytes may undergo feathery degeneration, and in prolonged cholestasis liver-cell rosettes are a common finding. Portal tracts usually remain normal but may be minimally inflamed. Because of the lack of necrosis and inflammation, this type of lesion is sometimes known as pure or bland cholestasis.

Differential diagnosis

The differential diagnosis is from other causes of bland cholestasis such as benign recurrent intrahepatic cholestasis, which is discussed in detail in **Chapter 5**.

Idiosyncratic drug-induced cholestasis

Idiosyncratic drug-induced cholestasis, typified by chlorpromazine jaundice[115] but also caused by many other drugs, differs from bland cholestasis in that some degree of portal inflammation is usually present (**Fig. 8.13**). There is sometimes inflammatory infiltration of the lobules and evidence of hepatocellular damage. Zimmerman and Ishak[116] therefore refer to this type of lesion as 'hepatocanalicular'. The portal infiltrate often includes eosinophils, and these are occasionally abundant, but their absence does not exclude a diagnosis of drug-induced hepatocanalicular cholestasis. Small interlobular ducts often show abnormalities such as irregular

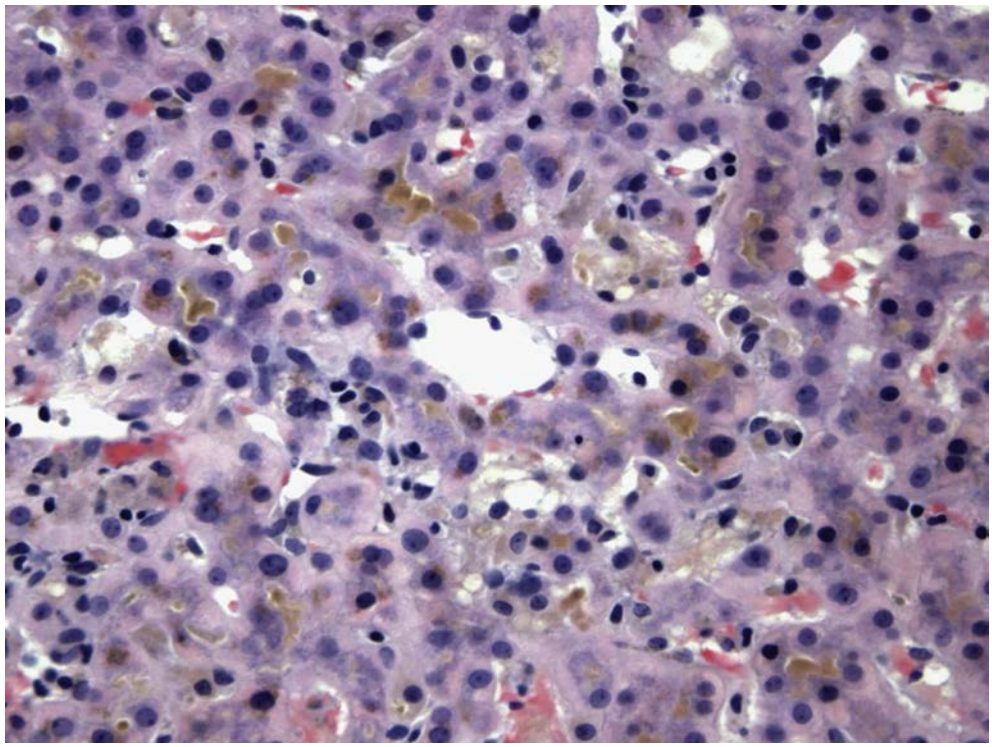

Fig. 8.12 Anabolic-androgenic steroid cholestasis. Bile canalicular cholestasis is prominent and accentuated near the efferent vein at centre. (Needle biopsy, H&E.)

distribution of epithelial cell nuclei, cytoplasmic vacuolation, variation in nuclear size and infiltration by lymphocytes. These changes are usually mild, but occasionally more severe (**Fig. 8.14**), even leading to ductopenia (see **vanishing bile-duct syndrome**, below). The lobular changes (**Fig. 8.13**) are as in bland cholestasis, except for the additional element of inflammation and necrosis which is sometimes found, as mentioned earlier. There is therefore a spectrum of appearances in this type of cholestasis, from an almost bland cholestatic lesion to one resembling mild acute viral hepatitis. Even in the absence of necrosis and inflammation, hepatocellular changes are seen, which possibly result from prolonged cholestasis itself, but which may also include an element of adaptive proliferation of the smooth endoplasmic reticulum. These changes include prominent hepatocellular swelling, abundant pale-staining cytoplasm and, commonly, multinucleation. Mitotic figures may be evident.[115]

Differential diagnosis

The differential diagnosis of idiosyncratic drug-induced cholestasis is from bile-duct obstruction, acute viral- or drug-induced hepatitis and cholestasis of the bland type. Portal oedema, prominent neutrophils, marked ductular reaction and absence of lobular inflammation favour the first. In the absence of substantial portal inflammation, the distinction between idiosyncratic drug jaundice and bland steroid-induced cholestasis becomes difficult to make and requires clinical information. In such circumstances bile-duct obstruction cannot be completely ruled out. The differential diagnosis also includes other causes of bland cholestasis, such as benign recurrent cholestasis (**Ch. 4**). Severe liver-cell damage and inflammation favour viral hepatitis or the drug injury of hepatitic type (already discussed).

The clinical course of idiosyncratic drug jaundice varies. In most patients removal of the offending drug leads to rapid improvement. Occasionally the cholestasis is slow to improve but liver biopsy shows cholestasis only, with no fibrosis or other evidence of progressive disease. In rare instances true chronic disease develops on the basis of severe bile-duct damage

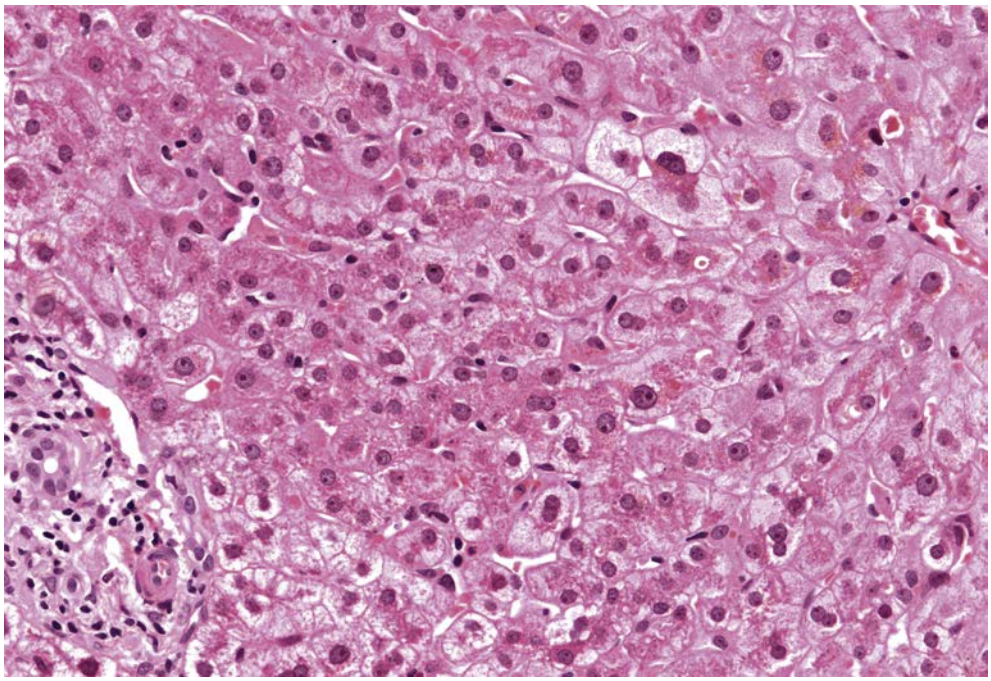

Fig. 8.13 Drug-induced liver injury: hepatocanalicular type. In this patient with jaundice following chlorpromazine therapy there is mild inflammation of the portal tract (lower left) and swelling of hepatocytes, especially in the perivenular area (above, centre and right). (Needle biopsy, H&E.)

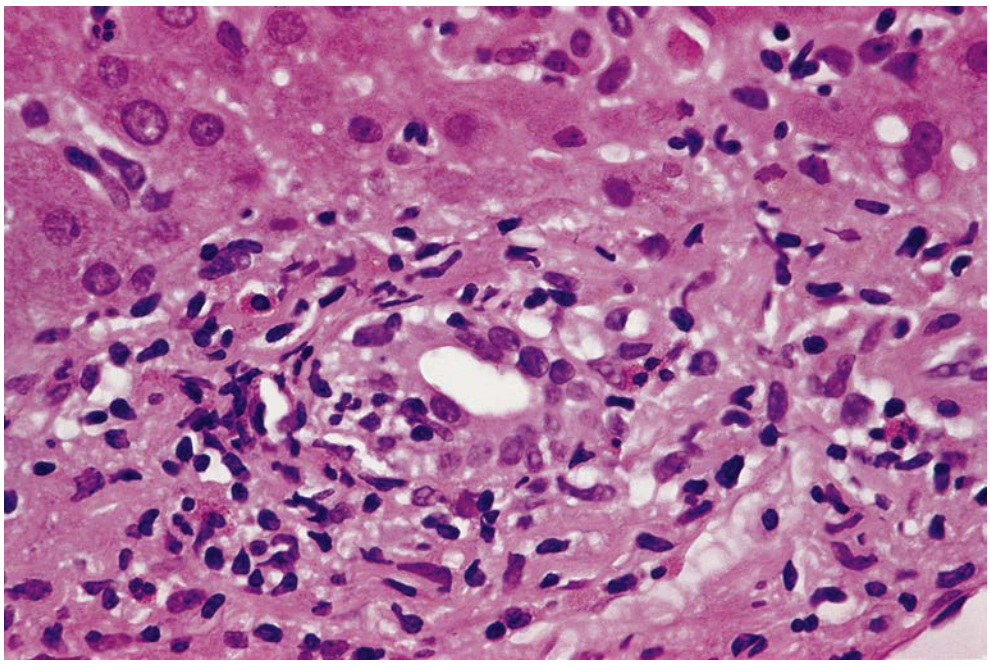

Fig. 8.14 Drug-induced liver injury: hepatocanalicular type. An inflamed portal tract from a patient with jaundice attributed to Augmentin (amoxicillin and clavulanic acid). The epithelium of an interlobular bile duct is irregular, vacuolated and infiltrated by lymphocytes. (Needle biopsy, H&E.)

and duct loss, with consequent fibrosis and other features of chronic biliary disease. The clinical picture resembles primary biliary cirrhosis. This **vanishing bile-duct syndrome** has been reported following a number of drugs,[117,118] including chlorpromazine,[115] a combination of chlorpropamide with erythromycin ethylsuccinate,[119] prochlorperazine,[120] gold salts,[121] ciprofloxacin,[122] haloperidol,[123] ajmaline,[124] glycyrrhizin[125] and amoxicillin and flucloxacillin,[126] among others. Augmentin (amoxicillin and clavulanic acid; **Fig. 8.14**) is a well-documented

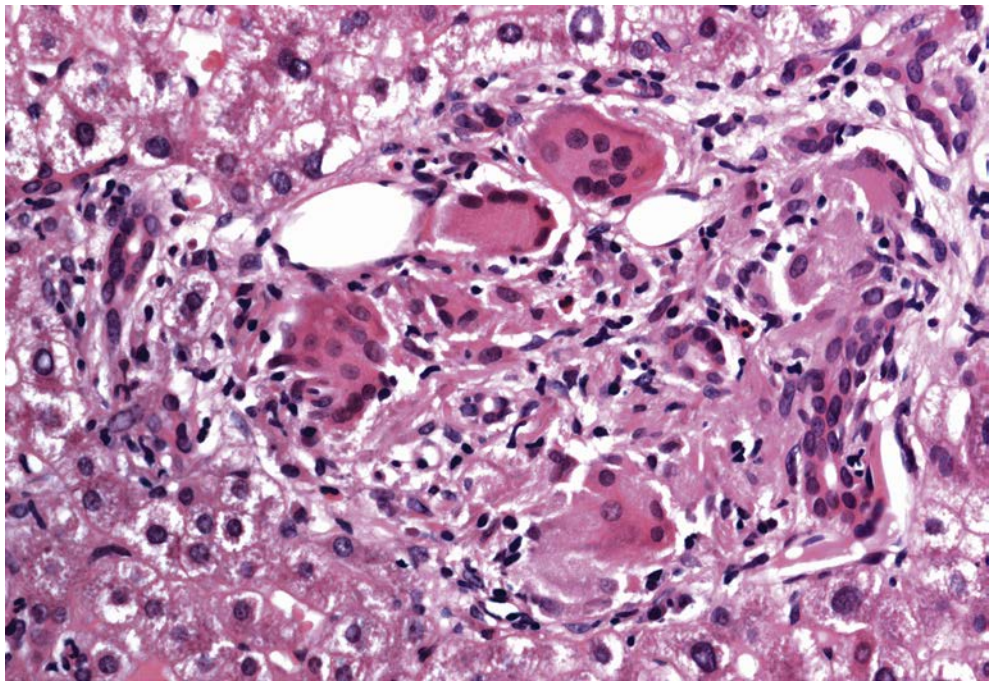

Fig. 8.15 Drug-induced granuloma formation. A portal tract contains a granuloma with many multinucleated giant cells. The patient became jaundiced after taking phenylbutazone. (Needle biopsy, H&E.)

cause of cholestasis, with striking focal destruction of bile ducts in some biopsies.[127–130] This is occasionally associated with granuloma formation. Prolonged cholestasis may be the result of the duct damage and, in some patients, of duct loss.

More acute bile-duct injury is seen in poisoning with the herbicide paraquat.[131,132] Zimmerman and Ishak[116] designate this type of injury as ductal or cholangiodestructive, in contrast to canalicular and hepatocanalicular cholestasis.

Long-term parenteral nutrition, already noted in relation to steatosis and steatohepatitis in adults, may be associated with a progressive form of liver injury in infants and children, typified by cholestasis, hepatocellular damage, ductular reaction, fibrosis and even cirrhosis.[58,59,133] Whether the parenteral nutrition itself is responsible for all these changes is not proven.[59,60] The lesion may mimic bile-duct obstruction[134] (**see Fig. 13.18**).

Granulomas

Drugs are an important cause of otherwise unexplained granulomas. They are sometimes the only or main manifestation of a drug reaction, but can also form part of a cholestatic or hepatitic picture.[103] The granulomas may be portal (**Fig. 8.15**), parenchymal or both. They usually show little or no necrosis, and are infiltrated by a variety of inflammatory cells, including plasma cells and eosinophils. Fibrin-ring granulomas have been reported with allopurinol[135] and, more recently, with immune checkpoint inhibitor cancer therapy. The list of drugs associated with hepatic granulomas is small compared with the list of those causing hepatitis or cholestasis, but it is nevertheless substantial.[116,136,137]

Other lesions

Drugs may produce other hepatic lesions such as **nodular regenerative hyperplasia,**[138] as described with the combined chemotherapy agents 5-fluorouracil and oxaliplatin.[139]

Veno-occlusive disease, sinusoidal obstruction syndrome (**see Fig. 16.23**) and obliterative venopathy are other consequences of chemotherapy.[140]

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CHAPTER 9

Chronic Hepatitis

Definition and causes

Chronic hepatitis is a common reason for persistently abnormal liver function tests[1] and forms the background for the development of much cirrhosis[2] and hepatocellular carcinoma. It is defined as persistence of liver injury with raised aminotransferase levels or viral markers for more than 6 months.[3] This definition, though artificial, helps to establish a borderline in studies of acute and chronic hepatitis. In practice, however, this line is not always easy to draw, because acute self-limiting hepatitis is sometimes prolonged beyond 6 months and chronic hepatitis may have an acute or indefinable onset. Many chronic liver diseases have an inflammatory component, but the term *chronic hepatitis* is often restricted to a limited number of causes (**Box 9.1**). The pathology of chronic hepatitis in the majority is fairly characteristic: the basic lesion is portal tract-based chronic inflammation, sometimes with variable degrees of periportal interface hepatitis and/or lobular necroinflammation. Features such as interface hepatitis and lymphocytic infiltration are sometimes seen in other conditions such as primary biliary cholangitis and primary sclerosing cholangitis, as discussed in **Chapter 5**. For the sake of clarity, a diagnosis of chronic hepatitis should therefore include the probable cause whenever possible.

Box 9.1 Classic causes of chronic hepatitis

Hepatitis B, with or without hepatitis D virus infection
Hepatitis C
Autoimmune hepatitis
Drug-induced hepatitis
Wilson's disease
Alpha-1-antitrypsin deficiency

Classification and nomenclature

The current classification is three tiered and includes designation of the *aetiology*, the *grade* of necroinflammation and the *stage* of fibrosis/cirrhosis. This classification replaces the obsolete terms 'chronic persistent hepatitis', 'chronic active hepatitis' and 'chronic lobular hepatitis'.[4,5] The primacy of aetiology in this classification is conceptually important, because the appearances on a given liver biopsy at any one time in chronic hepatitis reflect differing pathobiologic pathways such as viral kinetics or activity (or quiescence) of the immune system. This classification system is easily used in biopsy reporting in the form of single-line diagnosis, an example being '*Chronic hepatitis B with mild activity and mild periportal fibrosis (Grade 2, Stage 2)*'. The several systems available for semi-quantitative scoring of the grade and stage are discussed in detail at the end of the chapter.

Use of liver biopsy in chronic hepatitis

Box 9.2 Uses of liver biopsy in chronic hepatitis
Establishment of the diagnosis
Diagnosis of incidental lesions
Assessment of histological activity (grading)
Evaluation of types of necrosis
Evaluation of structural changes (staging)
Clues to aetiology and possible superinfection
Immunohistochemical assessment of viral antigens
Monitoring of therapy

Liver biopsy continues to play an important role in the diagnosis and management of patients with chronic hepatitis.[6–9] Biopsy may guide decisions on when to initiate or when to stop treatment[10] and, in patients with multiple aetiological agents, may help to establish their relative importance. Examples of the latter include the patient with chronic hepatitis C who has co-morbid risk factors for non-alcoholic fatty liver disease, or the patient with thalassaemia and viral hepatitis. Large-cell and small-cell change (dysplasia), possible predictors of hepatocellular carcinoma, are sometimes found before cirrhosis develops but will be discussed with the latter, in **Chapter 10**.

Box 9.2 lists the possible reasons for liver biopsy in chronic hepatitis.

Histological features of chronic hepatitis

Portal changes

Most small portal tracts are infiltrated to a variable extent by lymphocytes together with smaller numbers of plasma cells and occasional segmented leukocytes. A few eosinophils are often present. Lymphoid aggregates and lymphoid follicles with germinal centres are common in, but not exclusive to, hepatitis C. Larger conducting tracts are less affected than small terminal tracts and this has to be taken into account in assessing the severity of a hepatitis.

In the mildest forms of chronic hepatitis, the infiltrate is confined to portal tracts (**Fig. 9.1**) and the margins of the tracts remain regular. In the more severe forms, infiltration extends into the adjacent parenchyma, as will be described later. In mild chronic hepatitis, the tracts are often enlarged and short fibrous spurs may be seen extending from them (**Fig. 9.2**). These and other structural changes are most easily evaluated in reticulin or collagen stains. Interlobular bile ducts may be damaged, as shown by irregularity of the epithelial wall, vacuolation and infiltration by lymphocytes.

Parenchymal changes

The periportal lesion: interface hepatitis

In all but the mildest forms of chronic hepatitis, the inflammatory infiltrate extends from the portal tracts into the adjacent parenchyma and there is destruction of hepatocytes (**Figs. 9.3 and 9.4**). This process of interface hepatitis or piecemeal necrosis is most easily identified by the irregularity of the limiting plates of hepatocytes around the portal tracts. The term *interface hepatitis* is now often preferred to the older term 'piecemeal necrosis' because there is evidence to suggest that apoptosis rather than necrosis may be involved.[11,12] However, the relative roles played by apoptosis and necrosis in viral hepatitis are not yet entirely clear, because the two processes share several characteristics.[13]

Interface hepatitis at its mildest is recognised by lymphocytes in the periportal parenchyma, in association with hepatocellular damage. In more severe examples, trapped surviving hepatocytes may be seen within the inflammatory infiltrate (**Fig. 9.5**) and fibrous septa extend from the portal tract (**Fig. 9.6**). In cirrhotic livers the process is seen at the edges of nodules and septa rather than immediately around portal tracts (**see Fig. 10.20**);

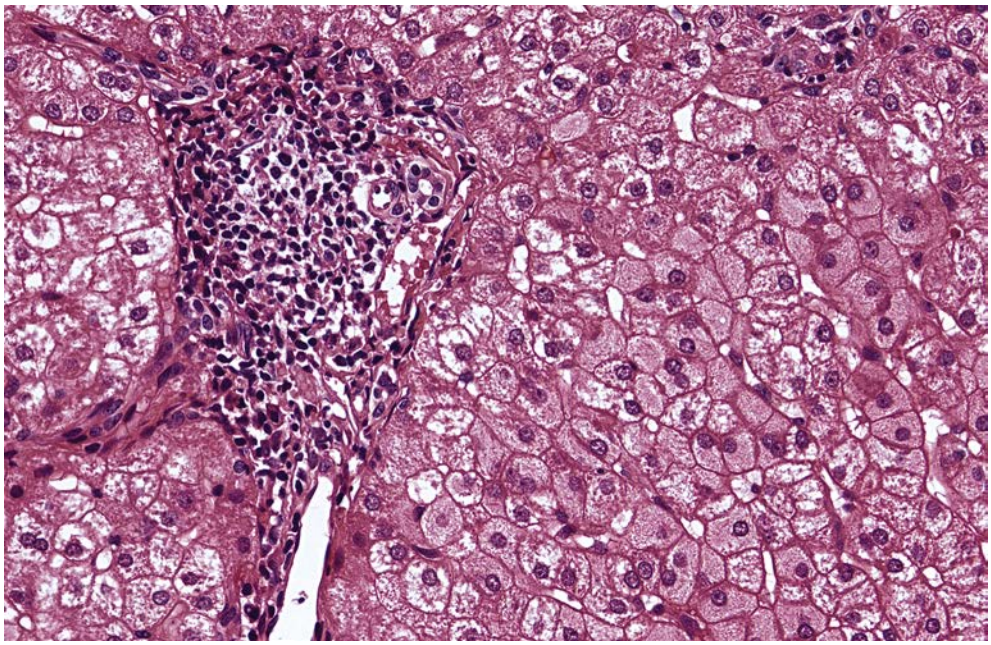

Fig. 9.1 Chronic hepatitis B, mild. The portal tract is heavily infiltrated with lymphocytes. These do not extend beyond the margins of the tract, the limiting plate of hepatocytes is intact and interface hepatitis is absent. Some hepatocytes have a ground-glass appearance. (Needle biopsy, H&E.)

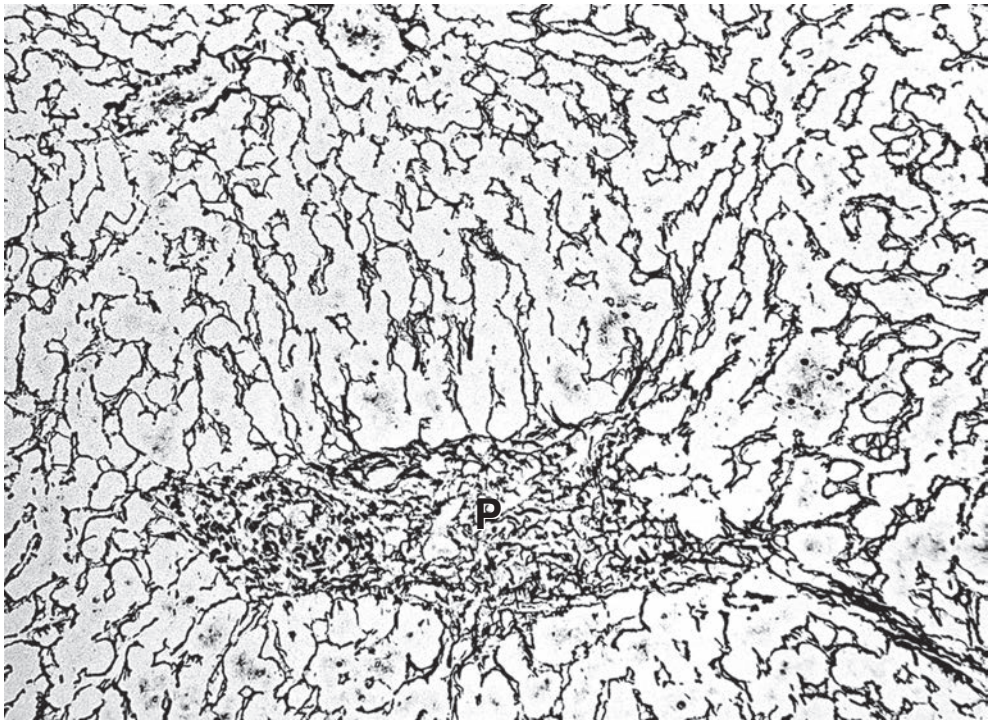

Fig. 9.2 Chronic hepatitis. Short septa extend from the slightly enlarged portal tract (P) but normal architectural relationships are preserved. (Needle biopsy, reticulin.)

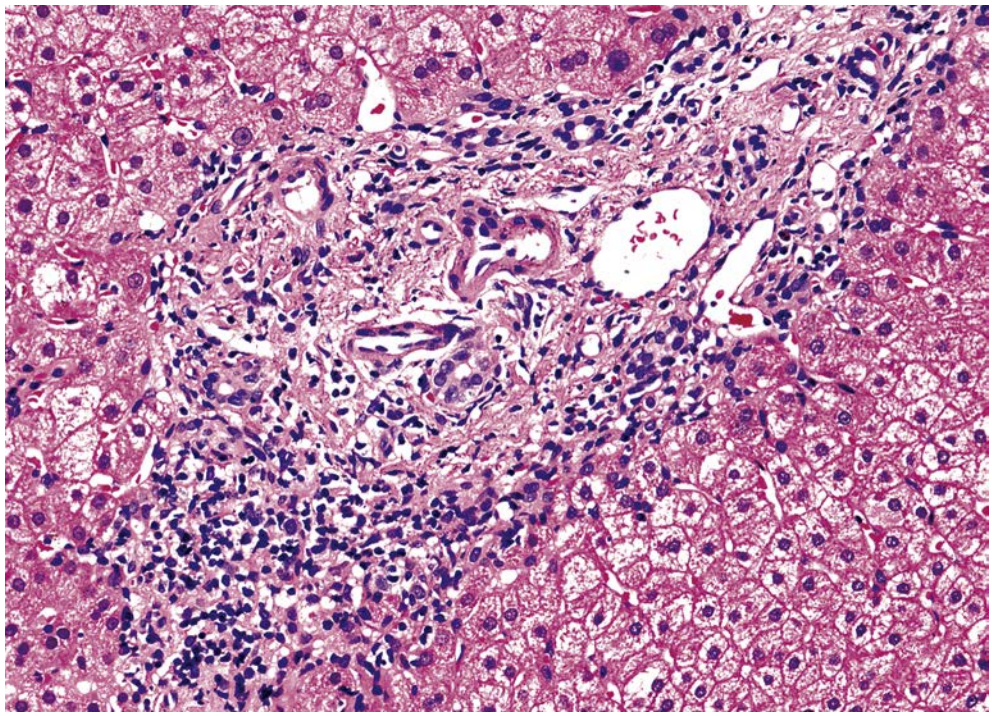

Fig. 9.3 Interface hepatitis. In contrast to the upper margin of this portal tract, the edges of the lower margin are blurred by inflammatory infiltration and hepatocyte loss. (Needle biopsy, H&E.)

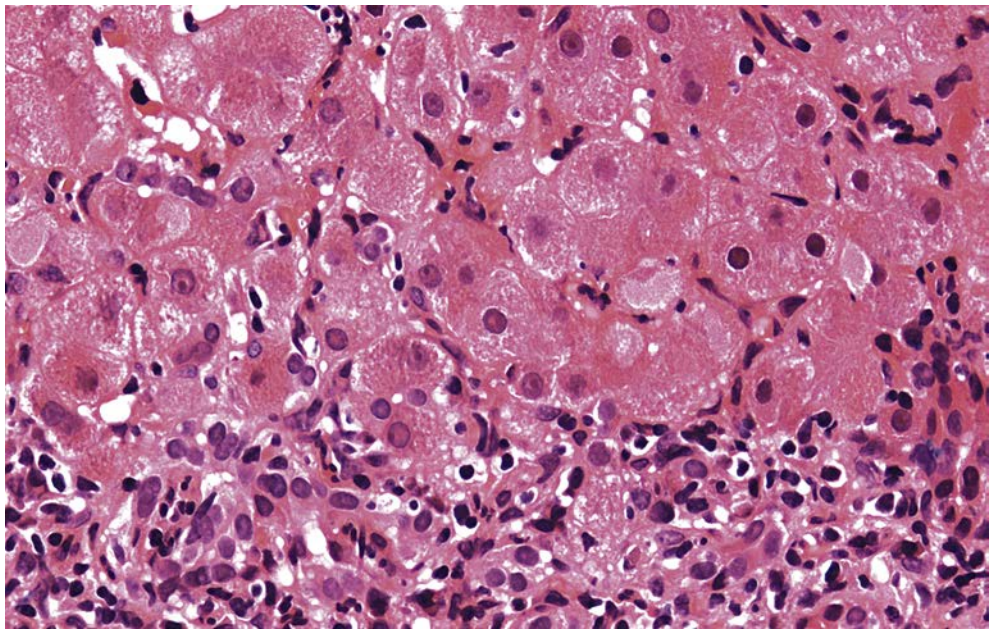

Fig. 9.4 Interface hepatitis. At a higher magnification than **Fig. 9.3**, lymphocytes are seen infiltrating between surviving hepatocytes. The interface between inflamed portal tract and parenchyma is irregular. (Needle biopsy, H&E.)

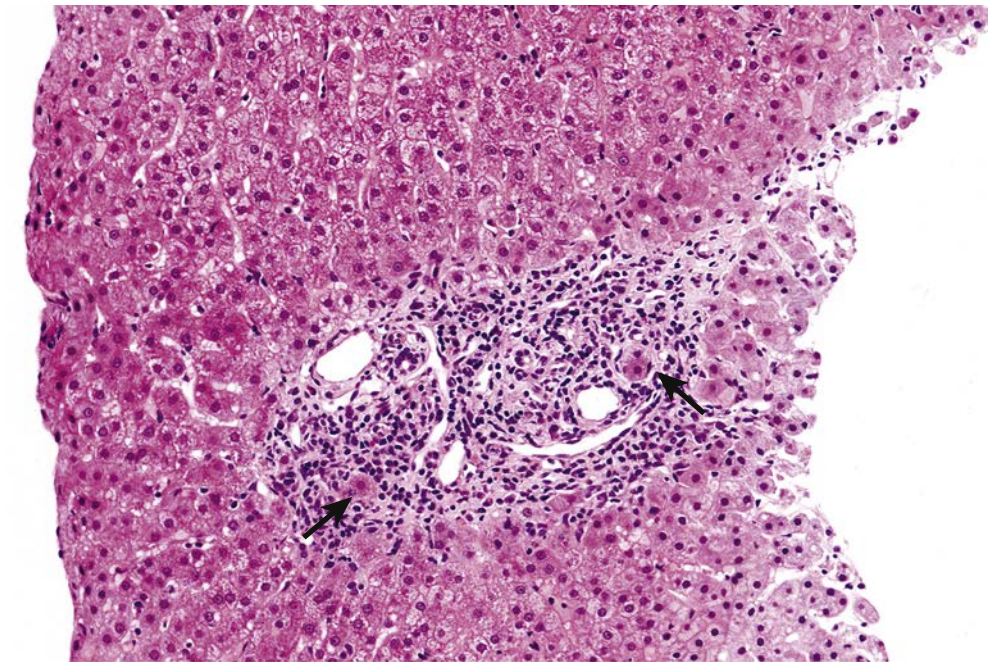

Fig. 9.5 Chronic hepatitis, mild to moderate. The lower edge of the portal tract shows interface hepatitis, with trapping of hepatocytes in the infiltrate (arrows). (Needle biopsy, H&E.)

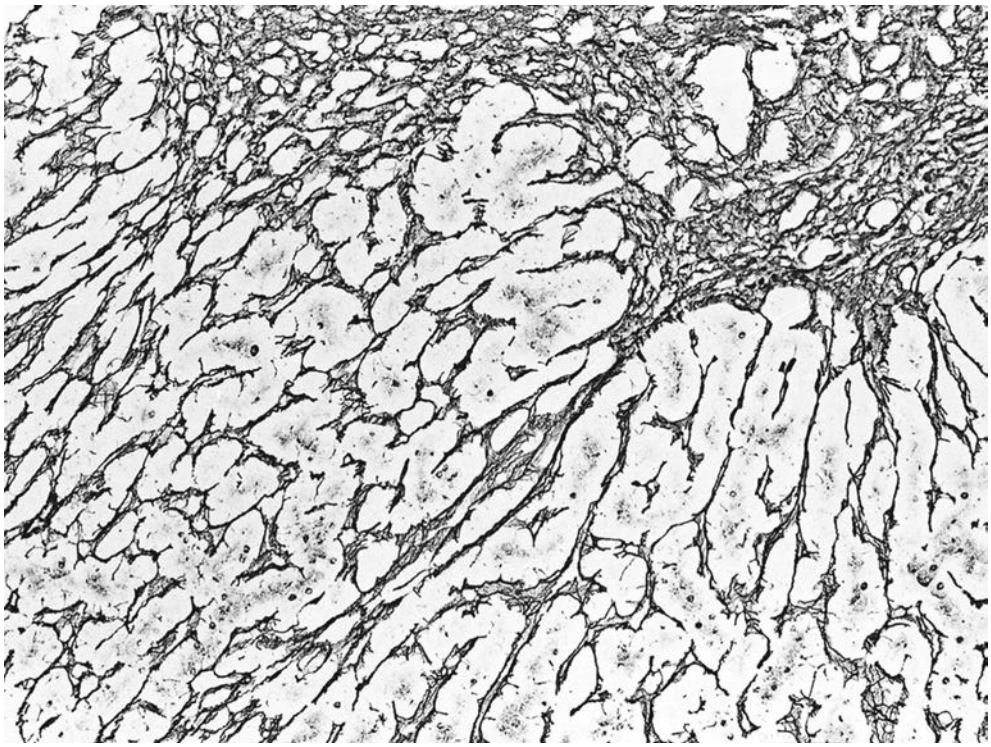

Fig. 9.6 Chronic hepatitis with fibrosis. Fibrosis extends from the portal tract above into the parenchyma. (Needle biopsy, reticulin.)

in either case, however, the hepatitic process involves the interface between connective tissue and parenchyma.

Interface hepatitis varies not only in severity but also in the extent of involvement of the interface, whatever its exact location. This is taken into consideration in some grading systems. With more severe interface hepatitis and liver-cell damage, periportal progenitor cells may become activated to produce a ductular reaction (proliferated bile ductules).[14] The blurring of the margins of the portal tracts in such instances then results from the combination of periportal chronic inflammatory cells and the ductular structures (**Fig. 9.7**). The presence of scattered neutrophils near the ductules should not be confused with biliary obstruction, cholangitis or a presumed drug reaction; they are normal constituents of the ductular reaction, mediated by cytokines expressed by the ductular cells.[15]

The lobular lesion

Deeper within the parenchyma there are varying degrees of hepatocellular damage and inflammation, sometimes called the lobular component or lobular hepatitis. Most commonly, this takes the form of focal necrosis, but confluent and bridging necrosis may also be seen. Panlobular necrosis is rare in chronic hepatitis. Also uncommon is the finding of severe lobular hepatitis in the absence of substantial portal and periportal inflammation.[16] The severity of lobular hepatitis correlates with the accumulation of progenitor cells.[17]

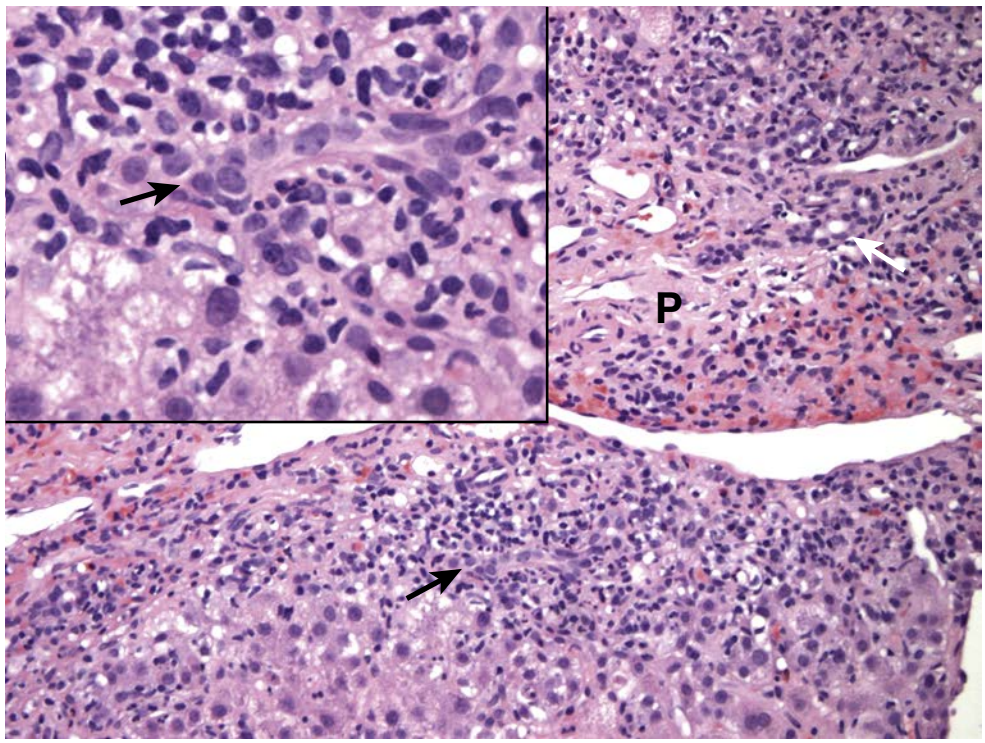

Fig. 9.7 Ductular reaction in chronic hepatitis with marked activity. The margins of the portal tract (P) above and below are expanded and effaced by marked interface hepatitis. The white arrow marks the native bile duct. Within the irregular border of interface hepatitis at bottom are bile ductular structures (ductular reaction), one of which (arrow) is enlarged in the inset. Inset: Scattered neutrophils surround and partially infiltrate the flattened ductule (arrow) in this area of lymphoplasmacytic interface hepatitis. (Needle biopsy, H&E.)

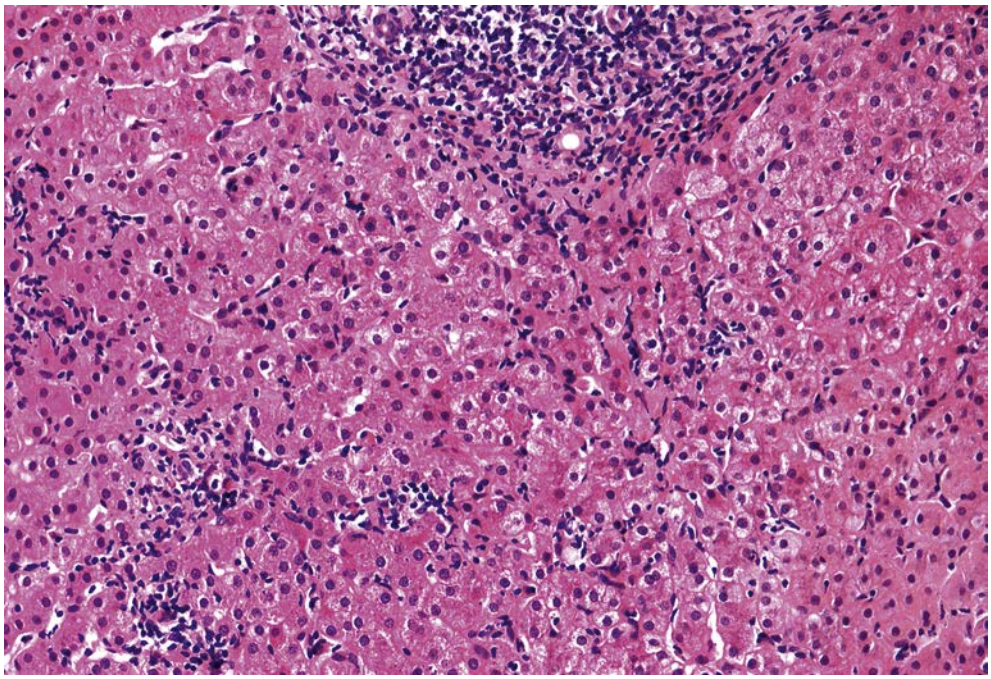

Fig. 9.8 Chronic hepatitis with lobular activity. Clumps of inflammatory cells, some of them associated with hepatocyte loss, extend through the parenchyma. The portal tract above is inflamed. (Needle biopsy, H&E.)

Focal (spotty) necrosis is seen as areas of hepatocyte loss with infiltration by lymphocytes, macrophages and other cells. Each area covers the space normally occupied by up to about four or five hepatocytes (**Fig. 9.8**). Larger areas of hepatocyte loss are referred to as confluent necrosis (**Ch. 4**). As in acute hepatitis, bridging necrosis refers to confluent necrosis and collapse linking vascular structures and is usually restricted to bridges linking portal tracts to terminal hepatic venules.

Severe lobular hepatitis is often accompanied by the formation of small rounded or ovoid gland-like clusters of surviving hepatocytes, so-called hepatitic rosettes (**Fig. 9.9**). Unlike cholestatic rosettes (**Ch. 4**), these are embedded in connective tissue and probably form as a result of hyperplasia of hepatocytes trapped in a collapsed and inflamed area of parenchyma.

In a minority of patients with chronic hepatitis some of the hepatocytes fuse to form multinucleated giant cells like those of neonatal hepatitis (**Fig. 9.10**). In adults this is termed *postinfantile giant-cell transformation*; it is an occasional feature of autoimmune hepatitis (AIH) and of chronic hepatitis C (with or without human immunodeficiency virus (HIV) co-infection),[18,19] typically present only in perivenular regions.

Other hepatocyte changes seen in chronic hepatitis include steatosis, iron deposition and oncocytic change. Steatosis is most common in chronic hepatitis C and is further discussed under that heading later, as is siderosis. Iron deposits are sometimes focal.[20] Substantial hepatocellular siderosis should always lead to consideration of possible hereditary haemochromatosis, but siderosis is not necessarily related to an *HFE* gene mutation.[21] Oncocytic change results from the accumulation of large numbers of closely packed mitochondria in hepatocytes, giving them a granular, densely eosinophilic appearance[22,23] (**Fig. 9.11**). These cells are most common within hepatitic rosettes. Mitochondrial hyperplasia in these cells appears to be a compensatory response to mitochondrial DNA dysfunction.[24] Finally, the appearance of bile thrombi in dilated canaliculi is most unusual in chronic hepatitis. While this type of cholestasis could result from an acute exacerbation of chronic disease, alternative explanations such as drug hepatotoxicity should be considered.

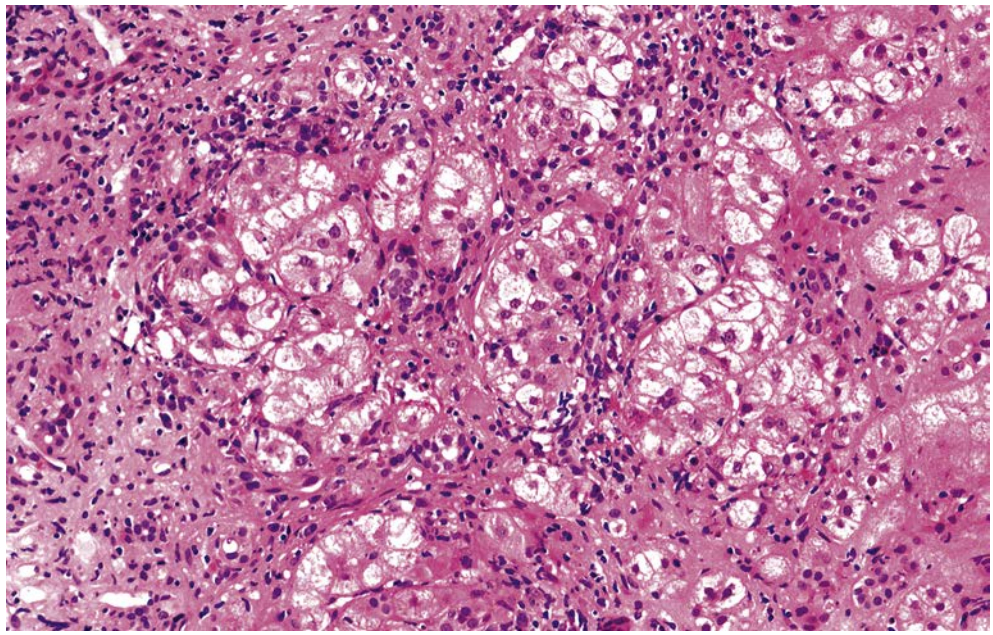

Fig. 9.9 Chronic hepatitis, severe, with rosette formation. Parenchymal architecture has been completely disrupted. Surviving hepatocytes have formed gland-like rosettes, which are separated by bridges of collapse and inflammation. (Needle biopsy, H&E.)

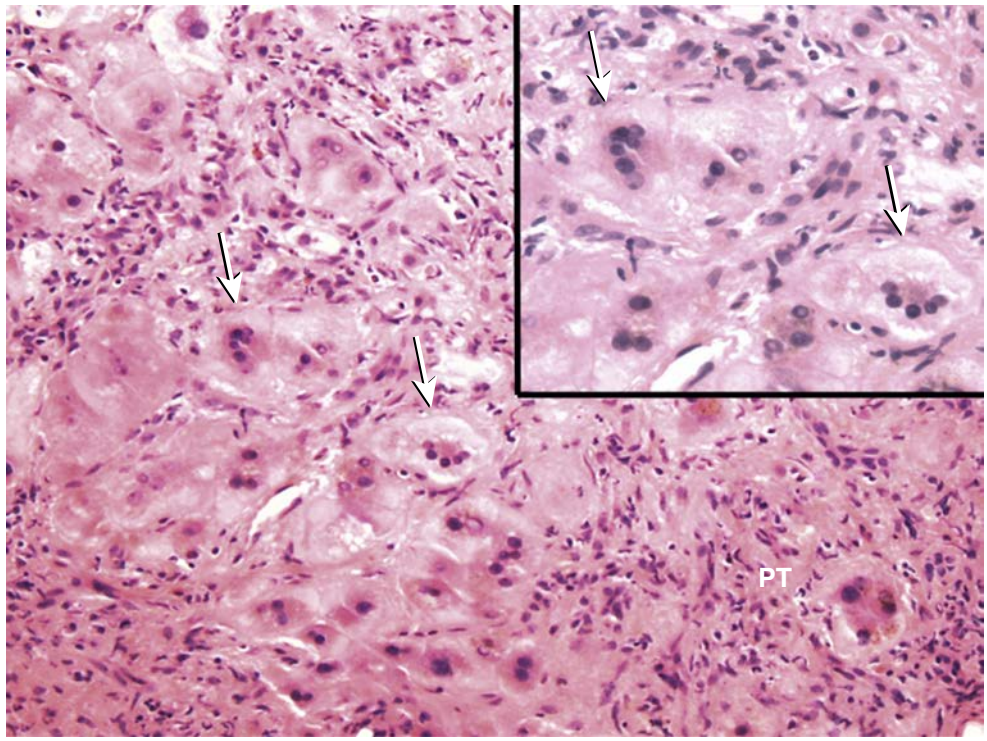

Fig. 9.10 Postinfantile giant-cell transformation in chronic autoimmune hepatitis. This patient had advanced autoimmune liver disease with cirrhosis and marked activity. Numerous multinucleated giant hepatocytes are present (arrows). Inset: Multinucleated giant hepatocytes show four or more nuclei in each cell (arrows). (Needle biopsy, H&E.)

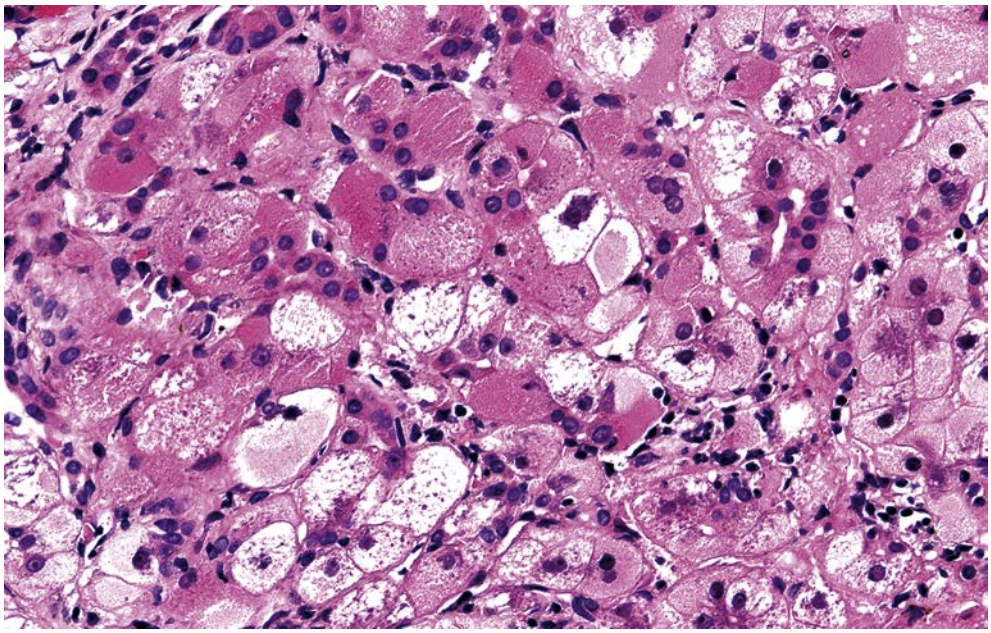

Fig. 9.11 Oncocyte formation in chronic hepatitis. Some of the hepatocytes in this severe chronic hepatitis have intensely eosinophilic granular cytoplasm. Others have a ground-glass appearance. (Needle biopsy, H&E.)

In some patients with AIH (and occasionally in patients with chronic viral hepatitis or with primary biliary cholangitis (PBC)), distinctive eosinophilic and diastase periodic acid–Schiff (PAS)-positive small globular inclusions known as *hyaline droplets* are seen in Kupffer cells within the sinusoids[25](**Fig. 9.12**). The inclusions have been shown to contain immunoglobulins (Igs), most often IgG, sometimes IgA and rarely IgD.[25]

Individual causes of chronic hepatitis

Chronic hepatitis B

Chronic hepatitis B infection in both adults and children[26,27] goes through a series of phases marked by different serological, histological and immunocytochemical findings.[28] It begins with a period of immune tolerance, in which there are high levels of hepatitis B virus (HBV)-DNA in serum. Hepatitis B e-antigen (HBeAg) is positive and anti-HBe negative. Histological activity varies, and both interface hepatitis and lobular hepatitis may be seen on liver biopsy. However, low levels of activity are more common. The surface antigen, HBsAg, is most abundant in the characteristic **ground-glass hepatocytes** (**Fig. 9.13**). The ground-glass cells are typically scattered singly throughout the parenchyma at this stage of infection. Their name derives from the finely granular appearance of the central part of the cytoplasm, which is rich in endoplasmic reticulum and hepatitis B surface material. Other organelles are located at the cell periphery and often appear to be separated from the ground-glass area by a pale halo. HBsAg can be demonstrated immunohistochemically (**Fig. 9.14**) and with orcein or Victoria blue methods. It is most abundant in the ground-glass hepatocytes, but can also be seen in a membranous or submembranous location in hepatocytes without a ground-glass pattern. The differential diagnosis of ground-glass hepatocytes is from the oncocytic cells described in the previous section, from drug-induced hypertrophy of the endoplasmic reticulum (**see Fig. 8.1**) and from inclusion-containing hepatocytes in cyanamide toxicity (**Ch. 8**), Lafora's disease, immunosuppressed transplant

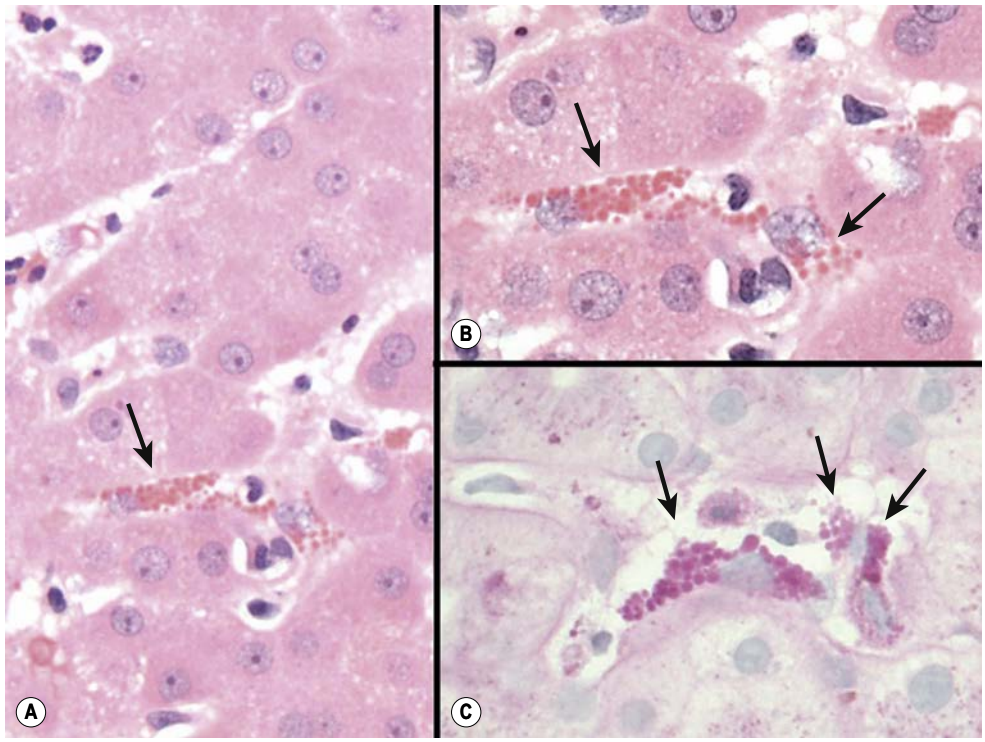

Fig. 9.12 Hyaline droplets (immunoglobulin) in Kupffer cells. A and **B:** Hyaline droplets resemble small Russell bodies and appear as discrete eosinophilic globules within the cell bodies of intrasinusoidal Kupffer cells (arrows). Their presence along with plasma cell–enriched inflammatory infiltrates favours a diagnosis of autoimmune hepatitis. **C:** The droplets are positively stained with diastase–periodic acid–Schiff (arrows). (Needle biopsy; **A** and **B:** H&E; **C:** diastase–PAS.)

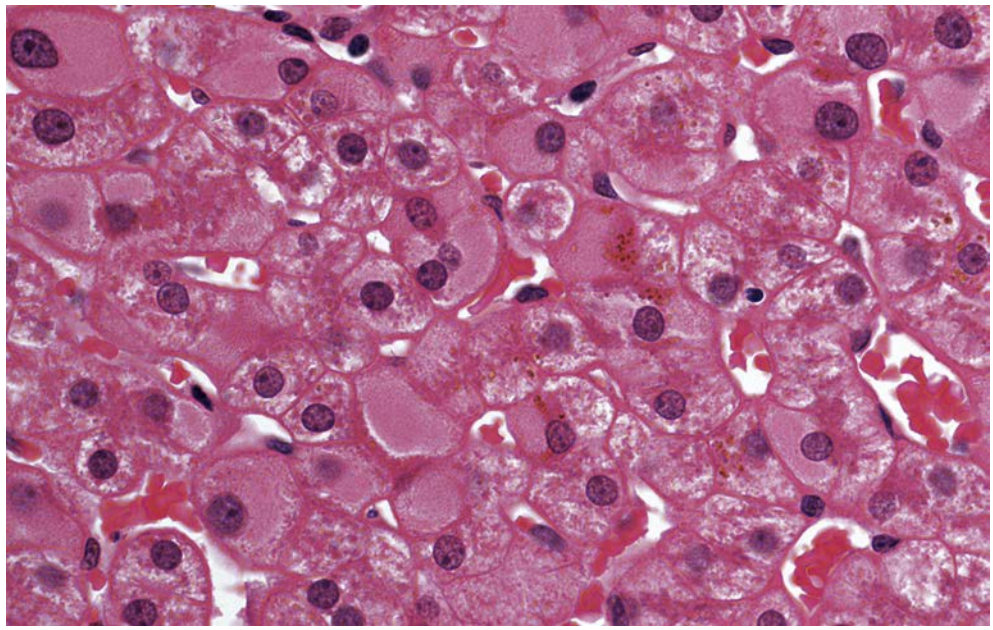

Fig. 9.13 Chronic hepatitis B with ground-glass hepatocytes. In many hepatocytes the central part of the cytoplasm has a homogeneous ground-glass appearance. A paler-staining halo is seen around the ground-glass areas in some cells. (Needle biopsy, H&E.)

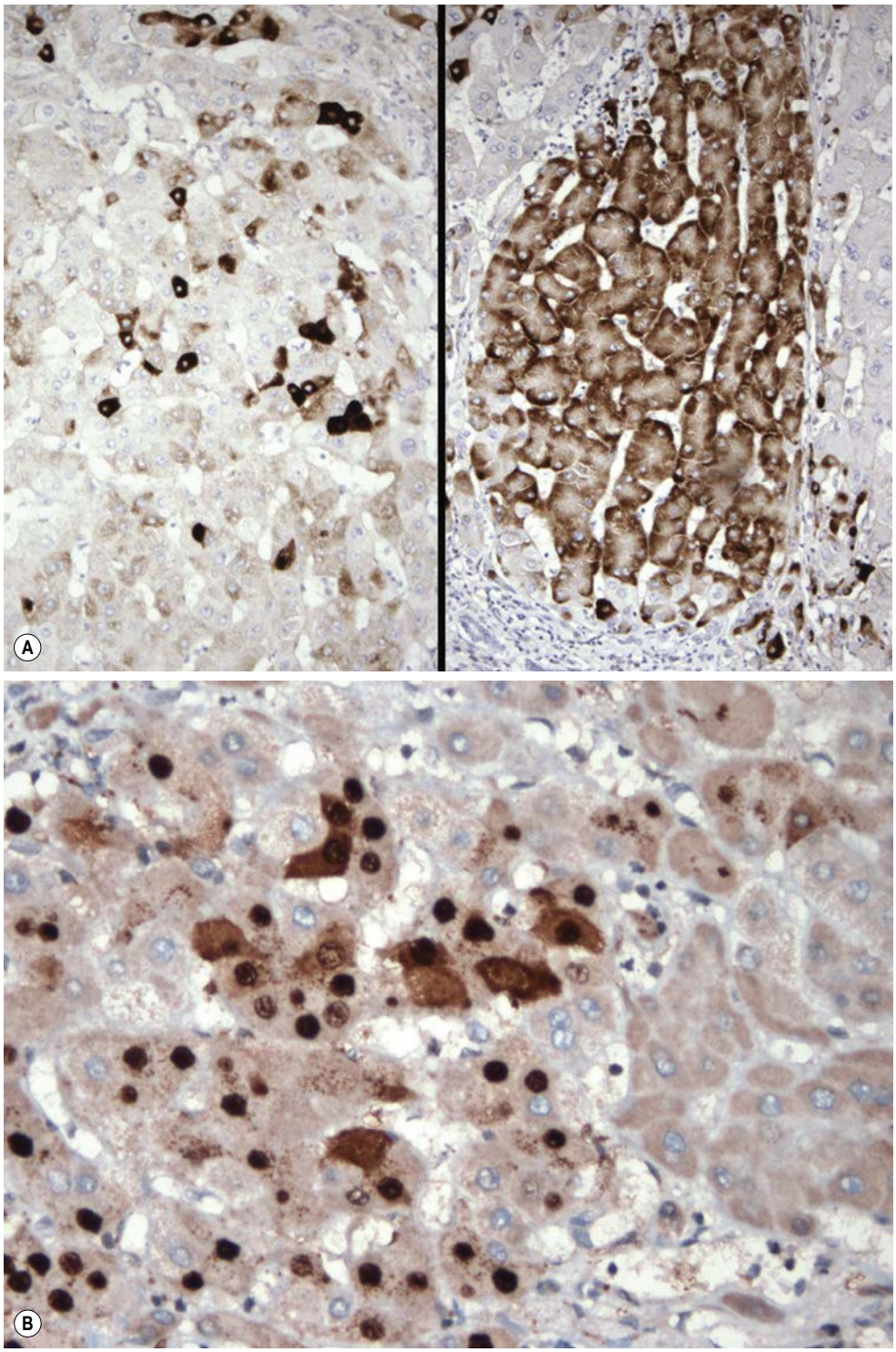

Fig. 9.14 Hepatitis B surface (HBsAg) and core (HBcAg) antigens with immunohistochemistry. A: Characteristic cytoplasmic inclusions of HBsAg are present within individual hepatocytes (left) or in groups of hepatocytes (right), sometimes referred to, respectively, as type I and II ground-glass hepatocytes. Type II ground-glass hepatocytes have been associated with pre-S2 gene deletions. **B:** Nuclear as well as cytoplasmic staining of HBcAg is present in this case, consistent with active viral replication. (Explant liver, specific immunoperoxidase stains.)

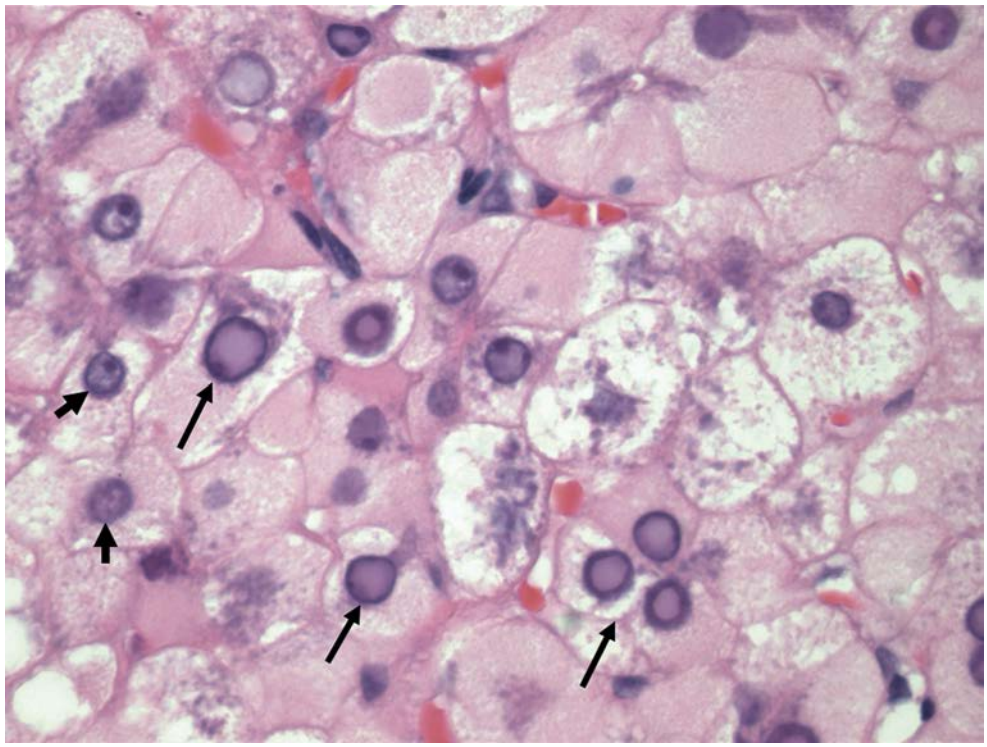

Fig. 9.15 'Sanded' nuclei with hepatitis B core antigen. In this case of chronic hepatitis B the pale homogeneous appearance of the affected hepatocyte nuclei (long arrows) reflects the presence of many intranuclear core particles. Several normal-appearing nuclei are in the field (short arrows). Many hepatocytes have ground-glass inclusions. (Needle biopsy, H&E.)

patients (**see** Fig. 4.4C and Ch. 16) and fibrinogen storage disease.[29] Clinical circumstances together with immunostaining for HBsAg make confusion unlikely.

The core antigen, HBcAg, is also demonstrable by immunostaining (Fig. 9.14). It is mainly located in hepatocyte nuclei, but also in cytoplasm when necroinflammatory activity is high. Positive nuclear staining correlates with viral load.[30] Nuclei which contain large amounts of core protein sometimes have a pale, homogeneous appearance on haematoxylin and eosin (H&E)-stained sections and have been described as 'sanded'[31] (Fig. 9.15).

The immunotolerant phase of chronic HBV infection is followed by immune clearance and seroconversion to a non-replicative phase in which HBeAg disappears from serum to be replaced by anti-HBe. During the phase of immune clearance, of very variable length, histological activity is typically high.

In the third, non-replicative phase, histological activity is usually considered to be low, as are markers of viral replication. However, in a large study of liver biopsies from patients in this phase, about one-third showed varying degrees of interface hepatitis, sometimes in the presence of normal aminotransferase levels.[32] Lobular activity was not necessarily accompanied by portal and periportal inflammation. Ground-glass hepatocytes may be clustered in focal accumulations in the late replicative or non-replicative phases. These clusters show dense marginal and/or submembranous HBsAg on immunostain (Fig. 9.14A), reflecting the presence of pre-S2 mutant forms of HBV with deletions in the gene encoding the pre-S2 envelope protein.[33,34] These deletions appear to confer protection from immunological attack as well as enhanced cell proliferative capacity favouring hepatocellular carcinoma.

Reactivation of virus replication and histological activity are common and may develop when chemotherapy[35a] or immunomodulatory agents are administered, or in association with the emergence of viral mutants. In some of these mutants, expression of HBeAg is defective and histological activity is unexpectedly high, in spite of the negative HBeAg

and presence of anti-HBe. Reactivation of hepatitis B after liver transplantation may have unusual histological features such as macrovesicular steatosis or more prominent diffuse fibrosis, colloquially referred to as 'steatoviral' or 'fibroviral' hepatitis B.[35b]

Finally, in a minority of patients with chronic HBV infection, HBsAg becomes negative and anti-HBs appears in the serum. HBV-DNA may still be detectable in small amounts in serum and liver.

This complex evolution, not always as orderly as the aforementioned simplified description might suggest, is marked by a very variable degree of fibrosis, depending on the severity and timing of the hepatitic process. Cirrhosis may develop at any stage, especially in patients whose HBV infection is complicated by infection with other viruses such as hepatitis C virus (HCV) and hepatitis delta virus (HDV).[36]

Apart from the presence of ground-glass hepatocytes and HBV antigens, there are other features which characterise chronic hepatitis B. Marked variation in the size and appearance of hepatocyte nuclei has been described,[37] as has close contact between hepatocytes and lymphocytes,[38] in keeping with the immunological nature of the hepatitis. The lymphocytes are usually of $CD8^+$ type, in contrast to the portal infiltrate, which is rich in $CD4^+$ lymphocytes, B lymphocytes and dendritic cells.[39] Lymphoid follicles are occasionally found in portal tracts but are less common and less prominent than in hepatitis C.[40]

Chronic hepatitis D (with HBV)

Infection with HDV modifies infection with HBV, as already noted in **Chapter 6**. Its presence is associated with relatively high histological activity except after liver transplantation. Inflammation is rarely restricted to portal tracts, and there is likely to be substantial inflammation in periportal areas as well as deeper within the lobules. Positive immunostaining for HDV (**see Fig. 6.17**) denotes active infection. A 'sanded' appearance similar to that produced by hepatitis core protein may be seen when there is abundant HDV in hepatocyte nuclei.[41] In the presence of HDV infection there is a greater risk of chronicity than with HBV alone, and liver-associated mortality is increased.[42] Once cirrhosis has developed in patients with hepatitis B, HDV infection confers a greater risk of developing hepatocellular carcinoma and a higher mortality.[43] The prevalence of HDV infection has declined since the 1970s and 1980s as a result of measures to eradicate HBV infection.[44] A prevailing HDV prevalence of approximately 8%–10% (based on seropositivity for antibodies to HDV) is seen in many parts of northern Europe, the UK and Japan, while regions of eastern Europe, the Middle East, Asia and Africa have higher prevalence rates, often in intravenous drug users.[44]

Chronic hepatitis C

The global prevalence of individuals with viraemic chronic HCV infection (i.e. serum HCV RNA-positive) has recently declined because of the availability of direct-acting antiviral agents (DAAs) for effective treatment. A recent modelling study found a global prevalence rate of 71.1 million people, with genotypes 1 and 3 the most common.[45] The success of DAA therapy with elimination of HCV from serum (sustained viral response) in the past several years has resulted in many pathology practices seeing a decline in numbers of liver biopsies obtained from HCV-positive individuals. Nonetheless, there remain many HCV-positive individuals who are untreated or were previously unsuccessfully treated for HCV infection, and pathologists should remain familiar with the histologic features of HCV-related liver disease. Chronic hepatitis C is not usually life-threatening until cirrhosis develops, typically several decades after onset of the hepatitis. Prior to available DAA therapy, factors associated with faster progression to cirrhosis included older age,[46] male sex, fibrosis on initial biopsy,[47] high necroinflammatory activity on initial biopsy,[48] iron deposition (see the 'Pathological features' section), alcohol consumption, previous HBV infection[49] and HIV infection.[50] There are six different genotypes of the virus,[51] and these affect disease severity.

Pathological features

Box 9.3 Histological features of chronic type C hepatitis
Difficult to distinguish from acute hepatitis C
Often mild, but cirrhosis commonly develops
Lymphoid follicles and/or aggregates in portal tracts
Damaged interlobular bile ducts
Lobular activity, including acidophil bodies
Large-droplet steatosis
Lymphocytes in sinusoids
Granulomas (uncommon)

The histological features of chronic hepatitis C, although not completely diagnostic in themselves, are very characteristic[40,52] (**Box 9.3**). The portal infiltrate is rich in lymphocytes which often form aggregates or follicles, some of them with prominent germinal centres (**Fig. 9.16**). These follicles are easily identified in reticulin preparations (**Fig. 9.17**). Follicles are not restricted to hepatitis C and can also be found in hepatitis B, AIH, primary biliary cirrhosis and primary sclerosing cholangitis, but in hepatitis C they are particularly common and prominent. Within, or to one side of, the lymphoid infiltrates, damaged interlobular bile ducts may be seen, as in acute hepatitis. The damage takes the form of vacuolation, stratification and crowding of epithelial cells, and infiltration by lymphocytes.[53] The virus has been demonstrated in bile-duct epithelium and in bile.[54] Bile-duct damage is occasionally, but by no means always, associated with a clinically cholestatic course, and rare ductopenia has been reported.[55,56]

The intralobular changes typically include acidophilic degeneration of hepatocytes and formation of acidophil bodies, already described in **Chapter 6**. Confluent necrosis is uncommon. Sinusoids are focally or diffusely infiltrated by lymphocytes, giving rise in some biopsies to a striking beaded appearance reminiscent of infectious mononucleosis. Epithelioid-cell granulomas and lipogranulomas are occasionally found in lobules or portal tracts,[57–59] and clumped material somewhat like Mallory–Denk bodies has been

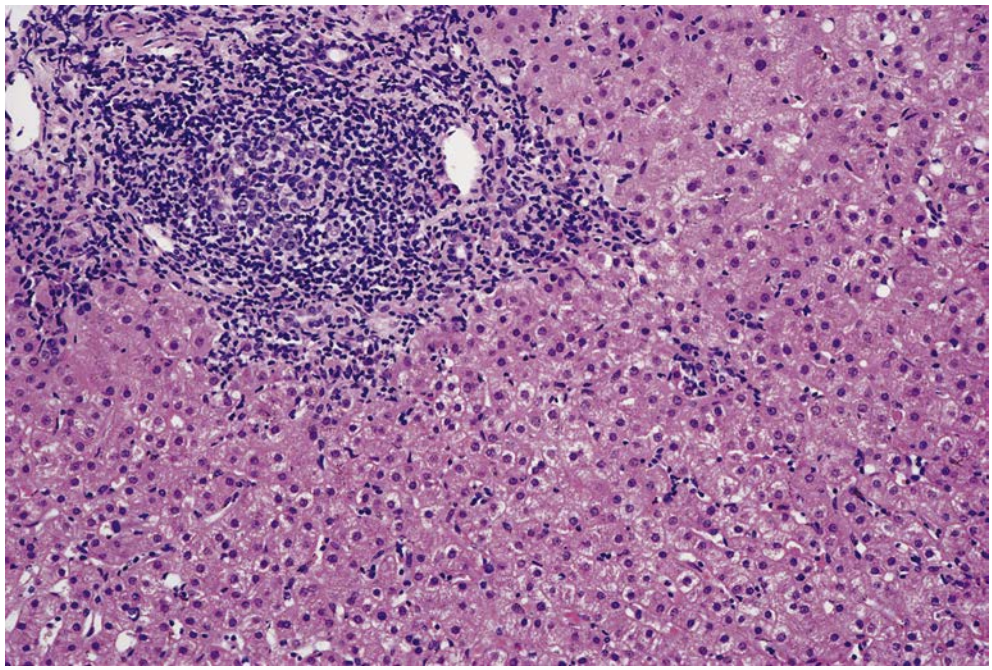

Fig. 9.16 Chronic hepatitis C. The portal tract (top left) is heavily infiltrated by lymphocytes, which extend irregularly into the adjacent tissue. A lymphoid follicle with germinal centre has formed. (Needle biopsy, H&E.) (Reproduced from Scheuer PJ, Ashrafzadeh P, Sherlock S, et al. The pathology of hepatitis C. *Hepatology* 1992;15:567–71.)

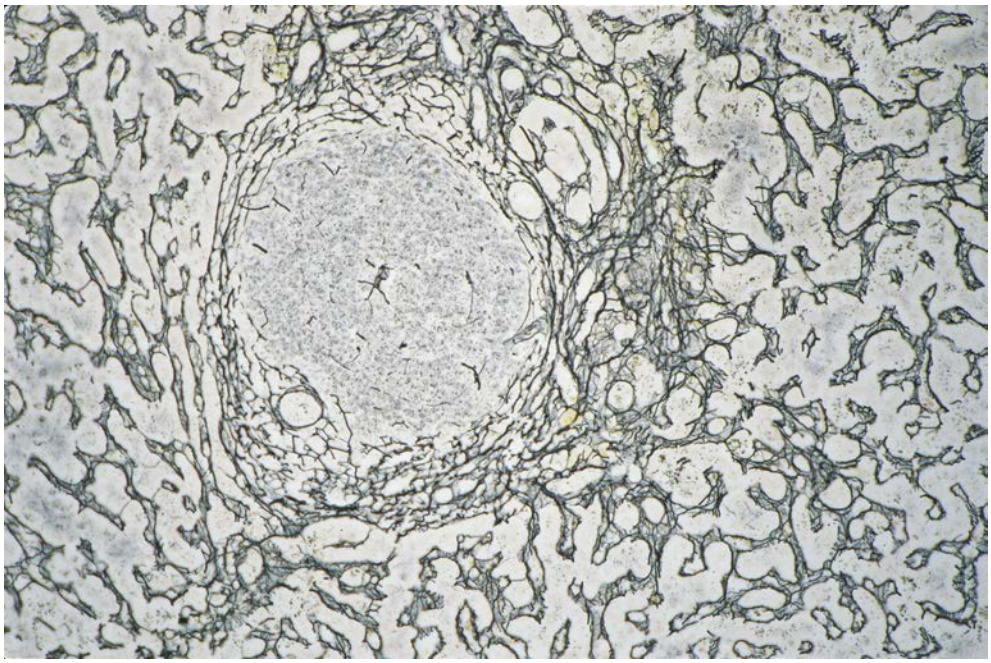

Fig. 9.17 Chronic hepatitis C. The prominent pale area in the portal tract is the site of a lymphoid follicle. (Needle biopsy, reticulin.)

reported in periportal hepatocytes.[60] The presence of talc crystals in liver tissue, seen by polarised light microscopy, is a specific but insensitive marker of intravenous drug abuse.[61]

Iron deposition is common even in the absence of the frequently found *HFE* mutations of hereditary haemochromatosis and may influence progression of the disease.[62,63] Iron is seen not only in hepatocytes but also in macrophages, endothelial cells and portal tracts.[64,65]

There is an extensive recent literature on the significance of steatosis in chronic hepatitis C. As already noted, steatosis is more common in hepatitis C than in other forms of chronic hepatitis and may be quite severe. It is a risk factor for progression,[66,67] and can interfere with therapy. The steatosis is often associated with obesity, diabetes or alcohol consumption.[57–71] However, in infection with HCV genotype 3[72,73] and very occasionally other genotypes,[74] the virus appears to have a direct effect and the steatosis improves after successful treatment.[75,76] The mechanism for the steatosis may be interference by the viral core protein with lipoprotein assembly and secretion.[77] In addition to steatosis, features of steatohepatitis such as pericellular fibrosis have been reported.[78] Polyarteritis nodosa is a rare complication of chronic hepatitis C.[79]

The development of reliable and clinically useful methods for detecting viral proteins by immunohistochemistry has been hampered by the small amounts of virus present in each cell, at least in immunocompetent patients. Although results using a monoclonal antibody against HCV envelope protein have been reported,[80] an immunostain for the identification of HCV in routine practice currently remains unavailable.

In biopsies taken early in the course of the disease, the hepatitis is often mild, with little interface hepatitis or fibrosis. With time, fibrous septa extend from expanded portal tracts and link vascular structures. Fibrosis linking portal tracts has the appearance of web-like membranes on three-dimensional reconstruction.[81] A pericellular pattern of fibrosis in perivenular areas has been reported in children.[82] Spontaneous clearance of virus[83,84] or specific treatment of the infection[85] may bring about dramatic improvement of the fibrosis and structural changes. Drug hepatotoxicity has recently been reported with administration of direct-acting antiviral HCV agents, resulting in an acute hepatitis characterised by focal lobular necrosis, portal and periportal eosinophils, lymphocytes and plasma cells.[86] Lastly, pathologists should be aware that explant liver specimens from HCV-positive individuals

with cirrhosis who have received direct-acting antiviral therapy prior to liver transplantation may continue to demonstrate histological features of mild chronic hepatitis, including persistence of lymphoid aggregates despite sustained viral response.[87]

Chronic hepatitis E

Hepatitis E virus (HEV) is recognised as the most common worldwide cause of acute viral hepatitis.[88] Oral–faecal transmission of HEV genotype 1 or 2 as a cause of serious acute hepatitis in developing regions of the world has been recognised for decades, but more recently concerns regarding HEV infection as a cause of chronic hepatitis, fibrosis and possibly cirrhosis have emerged, particularly in immunosuppressed individuals and in developed countries where zoonotic HEV genotypes 3 and 4 are transmitted from ingested meats or via blood products or haemodialysis.[89,90] Veterinarians and farmers are an at-risk population. Seroprevalence rates of IgG antibodies to HEV vary substantially worldwide, from low (approximately 6% in the United States[91]) to as high as 50% or more in regions of Europe and China.[92–94] Transmission of HEV in association with organ transplantation (heart,[95] lung,[96] kidney,[97] liver[98]) has occurred. The routine histopathology of chronic hepatitis E resembles that of chronic hepatitis B and C, with chronic portal inflammation and the potential for interface hepatitis, lobular necroinflammation, hepatocyte apoptosis, progressive fibrosis and cirrhosis.[99] However, in immunosuppressed individuals, there are recently described cases (predominantly in organ transplant recipients) where chronic portal and periportal inflammation is accompanied by excessive neutrophilic leukocytes and cholangitis. The bile duct damage is characterised by lymphocytic–neutrophilic infiltrates with epithelial changes resembling that seen in the florid bile-duct lesion of primary biliary cholangitis.[100] In suspected cases of chronic hepatitis E, serum assessment for HEV RNA and immunohistochemical staining for open reading frame 2 (ORF2; nuclear or nucleocytoplasmic positivity) should be pursued.[101–103]

Autoimmune hepatitis

AIH is an immune-mediated disorder in which hepatocytes are targeted and destroyed by a lymphocytic infiltrate accompanied, in its most histologically diagnostic form, by numerous plasma cells and occasional eosinophils. Individuals with AIH often have one or more other

Box 9.4 Conditions sometimes associated with features of autoimmune hepatitis

Drug hepatotoxicity (e.g. minocycline, nitrofurantoin)
Chronic hepatitis C
HIV disease with immune reconstitution
Transition from other autoimmune diseases (e.g. PBC)
Overlap syndromes (autoimmune hepatitis/PBC; autoimmune hepatitis/PSC)
After liver transplantation
Recurrent chronic hepatitis C
Recurrent chronic hepatitis C treated with interferon
De novo autoimmune hepatitis
Alloimmune late rejection

HIV, human immunodeficiency virus; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.

autoimmune disorders[104] (**Box 9.5**). AIH is diagnosed mainly on the basis of serum autoantibodies and absence of evidence for other causes of chronic hepatitis. The autoantibody profile is the basis for subclassification into different types.[105] The commonly assayed autoantibodies include anti-nuclear and anti-smooth-muscle antibodies (ANA and ASMA, respectively) and liver–kidney microsomal (LKM) antibodies. Other non-standard antibodies which may be present include soluble liver antigen (SLA), atypical peripheral antineutrophil cytoplasmic antibodies (atypical pANCAs) and anti-liver cytosol antibodies.[106–108] Antimitochondrial antibodies (AMAs) may be present in up to 35% of patients with otherwise typical AIH; they may persist for many years without clinical impact or evidence of primary biliary cholangitis.[109] Histological evidence is important not only for confirming the diagnosis but also as a means of detecting other conditions with which AIH may be confused. Histology is therefore one component of scoring systems which can be utilised in clinical practice.[110,111]

While there are no pathognomonic histological features of AIH, there is a characteristic picture in many patients before treatment. Biopsy shows active disease, with much hepatocellular damage and a heavy infiltrate of lymphocytes and plasma cells in portal tracts, at the interface and deep within the parenchyma (**Figs 9.18 and 9.19**). Plasma cells in clusters in interface regions are often striking. Eosinophils may also be present.[112] Lymphoid follicles are less prominent than in hepatitis C. Bridging necrosis is common, and surviving hepatocytes often form hepatitic rosettes (**Fig. 9.18**). Prominent syncytial giant hepatocytes in an adult hepatitis (**Fig. 9.10**), while not diagnostic, should always raise the possibility of AIH.[113,114] The presence of hyaline droplets[25] is an additional helpful diagnostic feature. Although Ig inclusions within hepatic sinusoidal cells were described as long ago as 1960 and 1969 by, respectively, Popper and colleagues[115] and Scheuer and colleagues,[116] and then again in the 1990,[117,118] their diagnostic value was only recently rediscovered in cases of paediatric AIH. These droplets resemble small versions of Russell bodies seen in plasma cells; the resemblance is further borne out on transmission electron microscopy.[119] Identification of hyaline droplets and plasma cell–rich inflammation (or plasma cells in clusters) in liver biopsy specimens appears to increase histological specificity for the diagnosis of AIH. Regenerative liver-cell rosettes and emperipolesis (lymphocyte entry into targeted hepatocytes), while often cited as features of AIH, are seen in other chronic

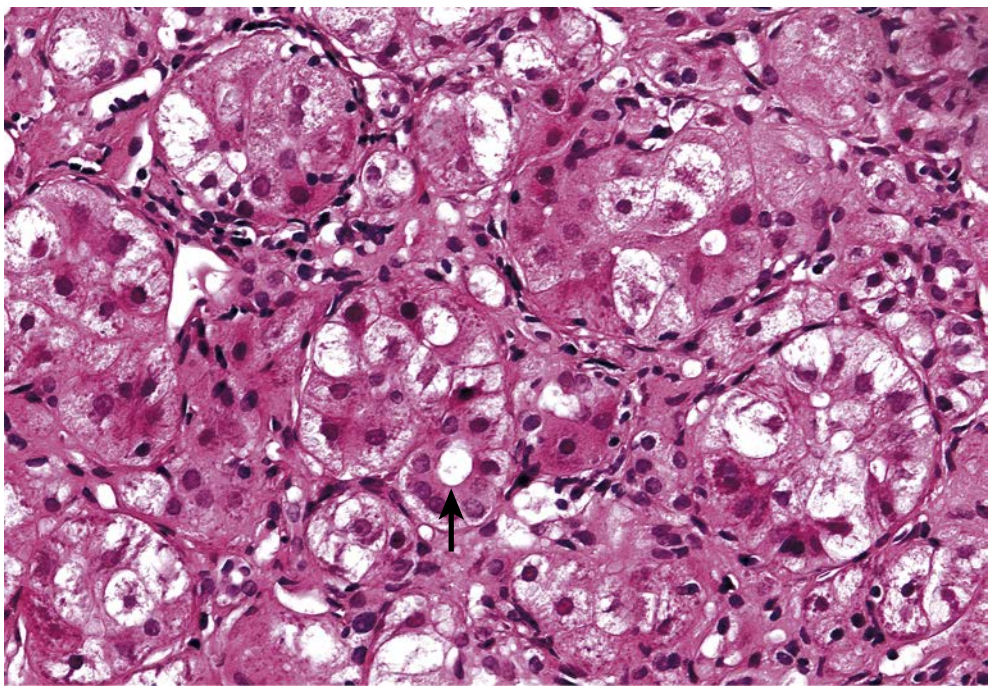

Fig. 9.18 Autoimmune hepatitis with rosette formation. Rounded hepatitic rosettes, some with a visible lumen (arrow), are surrounded by compressed sinusoids, fibrous tissue and inflammatory cells. (Needle biopsy, H&E.)

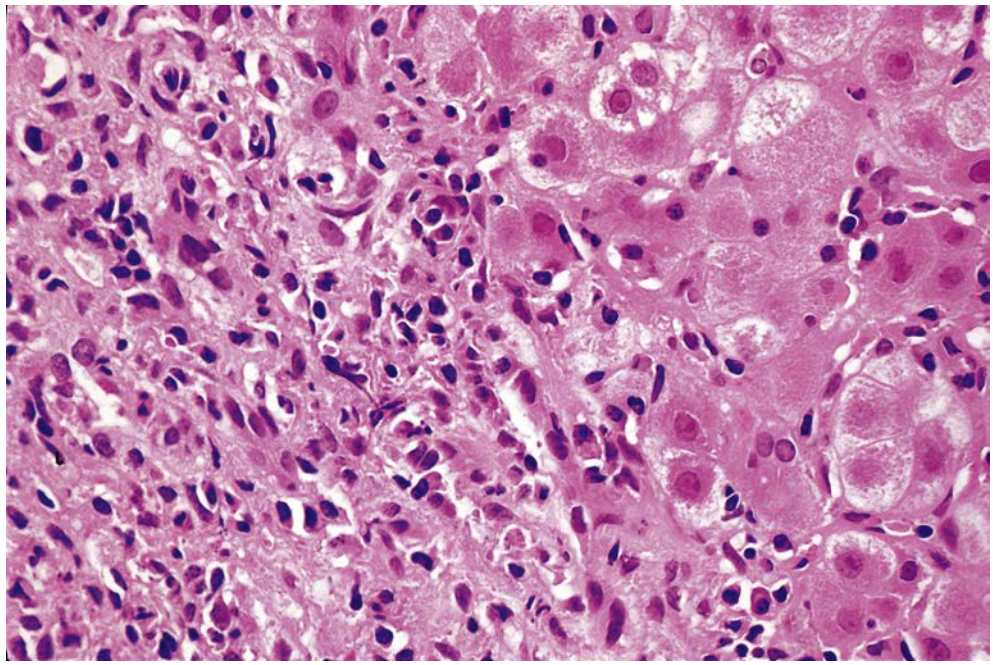

Fig. 9.19 Autoimmune hepatitis. Inflammatory cells including plasma cells extend from the portal tract (left) into the parenchyma as part of the process of interface hepatitis. (Needle biopsy, H&E.)

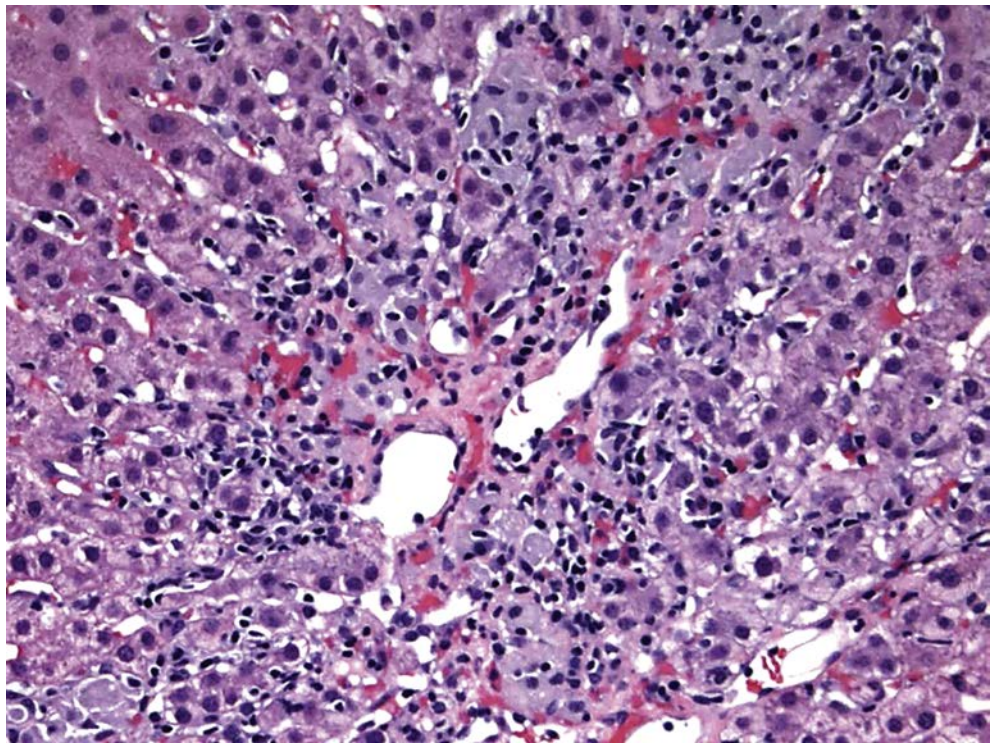

Fig. 9.20 Autoimmune hepatitis: histological variant form with centrilobular necrosis and inflammation. The liver parenchyma around the efferent vein (centre) shows hepatocyte drop-out and numerous inflammatory cells, including lymphocytes, plasma cells and clusters of tan ceroid-laden Kupffer cells. This type of centrilobular necroinflammation may be the only histological manifestation of autoimmune hepatitis, or may be present in combination with classical portal inflammation with interface hepatitis. (Needle biopsy, H&E.)

liver diseases as they progress towards cirrhosis (including alcoholic liver disease), and are therefore currently thought to bear insufficient specificity for diagnosing AIH.[120,121]

This classic histological picture is not, however, the only one seen in AIH, and communication between pathologist and clinician is important to ensure a correct diagnosis.[122] Plasma cells are not always present in large numbers. In some patients the hepatitis is much milder, and in some there may be cholestasis, bile-duct damage or even ductopenia.[123,124] In adults, this has to be distinguished from the bile-duct lesions of primary biliary cholangitis and the relatively uncommon overlap syndromes (**Ch. 5**). In children, AIH is associated with an autoimmune form of sclerosing cholangitis, autoimmune sclerosing cholangitis (Chapter 13).[125,126]

AIH is regarded as a chronic disease in all patients, but the clinical onset is sometimes acute. In a study of 26 patients biopsied within 6 months of onset,[127] most showed evidence of chronicity and a few had cirrhosis. However, careful analysis of connective tissue septa with the help of several connective tissue stains (**Ch. 6**) sometimes suggests recent onset with rapid development of nodules. Furthermore, there is a small subgroup of patients with a variant histological form of AIH characterised by centrilobular necrosis and inflammation, as in an acute hepatitis[128–131] (**Fig. 9.20**). The lesion is seen in perivenular regions where there are foci of hepatocyte drop-out and/or apoptosis, collections of lymphocytes with or without plasma cells and, typically, intrasinusoidal ceroid-laden Kupffer cells. The centrilobular necroinflammatory lesion may be the only histological manifestation of AIH, or it may be accompanied by the portal and periportal lymphoplasmacytic inflammation more typical of AIH. Some studies have suggested that the centrilobular necroinflammatory lesion is the early histological form of AIH which eventually progresses to a more classical chronic form of AIH based in portal and periportal regions. Exacerbation of disease severity may favour the development of confluence of the two patterns, resulting in central-to-portal bridging necrosis or even multilobular and massive necrosis.

Patients with AIH usually respond rapidly to corticosteroid therapy. Biopsy following treatment shows varying degrees of resolution of the necroinflammatory process and sometimes dramatic improvement in fibrosis and structural changes.[132,133] Liver biopsy helps to determine when corticosteroid treatment can safely be withdrawn.[10] The degree of plasma-cell infiltration is a predictor of relapse,[134] and worsening histological activity appears to correlate with progression of fibrosis.[135] Biopsy therefore continues to play an important role in patient management.

Other conditions with features of autoimmune hepatitis

Histological changes closely resembling AIH may occur in other settings (**Box 9.4**), sometimes accompanied by positive serum autoantibodies and elevated γ-globulin levels. The microscopic changes and generation of autoantibodies may be triggered by viral infection (chronic hepatitis C with autoimmune features[136]), drugs with idiosyncratic effects (nitrofurantoin and minocycline,[137,138] statins,[139,140] diclofenac,[140] black cohosh[141]) or medication-related immunomodulatory changes in an immunosuppressed individual [immune reconstitution after antiretroviral therapy in HIV disease[142]; after liver transplantation (**Ch. 16**)]. In such cases, the interpretation of AIH-like features merits clinical discussion along with review of current or recently changed medications. The type and titre of autoantibodies, γ-globulin level and the biopsy findings need to be factored into specific changes in management.

The potential for AIH to 'overlap' with other liver diseases of autoimmune nature, such as primary biliary cholangitis and primary sclerosing cholangitis,[143] was discussed in **Chapter 5**. Possible scoring systems for such overlap cases have been suggested recently.[144] Infrequently, one autoimmune disease may transition over time to another, such as primary biliary cirrhosis evolving to AIH. The transition may be evident as heightened necroinflammation, including interface hepatitis and bridging necrosis.[145,146] Because patients with overlapping features are not considered to represent specific or distinct clinical disorders by the International Autoimmune Hepatitis Group,[147] pathologists should commensurately 'toe the line' whenever possible and utilise the body of histological evidence to determine

one predominant diagnosis, possibly adding a diagnostic phrase to accommodate atypical features. For example, a biopsy from a middle-aged woman with positive serum anti-mitochondrial antibody that shows several stage 1 florid bile-duct lesions with granulomas diagnostic of primary biliary cholangitis, but also shows excessive interface hepatitis, could receive a diagnosis of 'Stage 1 primary biliary cholangitis, with autoimmune features'. Conversely, a patient with high-titre serum anti-nuclear antibody and a liver biopsy with extensive lymphoplasmacytic interface hepatitis, but also with an occasional damaged (but not destroyed) bile duct, would receive a diagnosis of 'chronic hepatitis with marked activity, consistent with AIH, with focal bile-duct damage'.

Differential diagnosis of chronic hepatitis

In biopsies with inflammation confined to portal tracts, other possibilities to be considered include **resolving acute hepatitis**, **non-specific inflammation near a focal lesion**, **primary biliary cirrhosis** and **lymphoma**. The nature of the infiltrate and involvement of most or all portal tracts in chronic hepatitis should resolve the issue in most cases, but clinical information is also needed.

More severe chronic hepatitis needs to be distinguished from **acute hepatitis**, which is sometimes difficult. As discussed in **Chapter 6**, staining for elastic fibres may enable recently formed bridges to be distinguished from old fibrous septa. Canalicular cholestasis, common in acute hepatitis, is not often found in chronic hepatitis. In HBV infection in an immunocompetent patient, the presence of HBsAg-containing ground-glass hepatocytes indicates chronic disease.

Other diseases to be considered in the more severe forms include **chronic biliary diseases**, especially primary biliary cholangitis and primary sclerosing cholangitis, **α_1-antitrypsin deficiency**, **Wilson's disease**, **lymphoma** and **drug injury**. Loss of bile ducts and periportal accumulation of copper-associated protein suggest biliary disease rather than chronic hepatitis. α_1-Antitrypsin deficiency can be diagnosed by appropriate staining (**Ch. 13**), while Wilson's disease (**Ch. 14**) should be established principally by clinical features and biochemical findings. The infiltrates of various lymphomas are usually extensive and irregular and may undergo necrosis. Drugs sometimes cause a liver disease closely resembling AIH, or may act as a trigger, unmasking latent autoimmunity, as discussed earlier. As in all liver diseases, correlation of clinical and histological findings reduces the risk of diagnostic error.

Semi-quantitative scoring: grading and staging

Scoring is now widely used to evaluate liver biopsies before treatment, to monitor the effects of treatment and to assess the effects of new therapies in clinical trials. It consists of two components, their names borrowed from oncology: **grading** and **staging**. Grading refers to the scoring of the necroinflammatory lesion of a hepatitis, including the various types and degrees of hepatocellular damage and the location and extent of the inflammatory process. Staging records the extent of fibrosis and of changes in structure, including the development of cirrhosis. In many scoring systems, grading is subdivided into categories such as portal inflammation, interface hepatitis and lobular hepatitis, whereas staging is expressed as a single scale.

Assessment of fibrosis can also be carried out using morphometric measurement of collagen.[148,149] This gives an accurate measurement of the amount of fibrous tissue per unit area, but does not take structural changes such as nodule formation into account. Staging and morphometry should therefore be viewed as complementary to each other and not as alternatives.

Scoring is semi-quantitative rather than quantitative, in the sense that while scores are usually expressed as numbers, they do not represent measurements. Scoring involves subjective

assessment of the various relevant histological features in a biopsy, and the scores allotted will inevitably vary somewhat from observer to observer depending on experience and personal bias. For this reason, scores allotted at different times or by different observers cannot be directly compared. This limits the usefulness of scoring as a routine reporting procedure.

Before embarking on scoring, the pathologist should consider carefully why the scores are required. This will help to determine the most suitable system for the particular purpose or project. For example, if what is needed is a decision as to whether the chronic hepatitis in a particular patient is mild, moderate or severe, a simple system will suffice, and will usually have the advantage over more complex systems in so far as the latter tend to be associated with greater intra- and interobserver variation and are also more time-consuming. If, by contrast, the purpose is to evaluate a group of biopsies in a clinical trial of a new treatment regime, then a complex system is more appropriate. A complex system would allow analysis not only of the overall severity of the changes, but also of individual features such as interface hepatitis and lobular activity. Examples of two simple systems are given in **Boxes 9.6 and 9.7**.[150,151] The simple staging system proposed by the METAVIR group[152] is given in **Box 9.7** and in **Table 9.1**, and the more complex and widely used Ishak system,[152] derived from the earlier Knodell Histology Activity Index,[153] in **Table 9.2**. Examples of grading and staging are shown in **Figs 9.21 and 9.22.**

Box 9.5 Autoimmune disorders associated with autoimmune hepatitis

Thyroiditis
Rheumatoid arthritis
Sjögren's syndrome
Systemic lupus erythematosus
Coeliac disease
Inflammatory bowel disease
Multiple sclerosis

Box 9.6 A simple scoring system for chronic hepatitis

Grade

Portal inflammation and interface hepatitis

0 Absent or minimal
1 Portal inflammation only
2 Mild or localised interface hepatitis
3 Moderate or more extensive interface hepatitis
4 Severe and widespread interface hepatitis

Lobular activity

0 None
1 Inflammatory cells but no hepatocellular damage
2 Focal necrosis or apoptosis
3 Severe hepatocellular damage
4 Damage includes bridging confluent necrosis

Stage

0 No fibrosis
1 Fibrosis confined to portal tracts
2 Periportal or portal–portal septa but intact vascular relationships
3 Fibrosis with distorted structure but no obvious cirrhosis
4 Probable or definite cirrhosis

Modified from Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* 1991;13:372–374.

Box 9.7 The METAVIR staging system

F0 No fibrosis
F1 Stellate enlargement of portal tracts but without septum formation
F2 Enlargement of portal tracts with rare septum formation
F3 Numerous septa without cirrhosis
F4 Cirrhosis

Modified from Bedossa P, Bioulac-Sage P, Callard P, et al. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994;20:15–20.

Table 9.1 The METAVIR algorithm

Interface hepatitis* (piecemeal necrosis)		Lobular necrosis†		Overall histological activity‡
0	+	0	=	0
0	+	1	=	1
0	+	2	=	2
1	+	0	=	1
1	+	1	=	1
1	+	2	=	2
2	+	0	=	2
2	+	1	=	2
2	+	2	=	3
3	+	0	=	3
3	+	1	=	3
3	+	2	=	3

*Interface hepatitis scored 0 (none), 1 (mild), 2 (moderate), 3 (severe).
†Lobular necrosis scored 0 (none or mild), 1 (moderate), 2 (severe).
‡Histological activity scored 0 (none), 1 (mild), 2 (moderate), 3 (severe).
Modified from Bedossa P, Poynard T, the METAVIR cooperative study group. An algorithm for the grading of activity in chronic hepatitis C. *Hepatology* 1996;24:289–293.

The results of a particular study can be compared in a general way with those of another, but because of the subjective nature of scoring, the numbers themselves cannot be directly compared or combined. Each study therefore stands on its own to some extent, and the observers are free to modify a published scoring system to suit a particular purpose. For instance, a scoring range for steatosis, siderosis or bile-duct damage could be devised and added if required.

Reproducibility of scoring is improved when it is performed by more than one observer.[154] There should then be an initial discussion using a multiheaded microscope in order to ensure that all observers agree on the criteria used to score each feature. At the end of a study, discrepancies between observers can be resolved by joint discussion at the

Table 9.2 The Ishak scoring system

Category	Score
Grading	
Periportal or periseptal interface hepatitis	
Absent	0
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around <50% of tracts or septa)	3
Severe (continuous around >50% of tracts or septa)	4
Confluent necrosis	
Absent	0
Focal	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis + occasional portal–central bridging	4
Zone 3 necrosis + multiple portal–central bridging	5
Panacinar or multiacinar necrosis	6
Focal (spotty) lytic necrosis, apoptosis and focal inflammation*	
Absent	0
<2 foci per 10× objective	1
2–4 foci per 10× objective	2
5–10 foci per 10× objective	3
>10 foci per 10× objective	4
Portal inflammation	
None	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked, all portal areas	3
Marked, all portal areas	4
Staging	
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal–portal bridging	3
Fibrous expansion of portal areas with marked bridging (portal–portal and portal–central)	4
Marked bridging (portal–portal and/or portal–central) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6

*Does not include diffuse sinusoidal infiltration by inflammatory cells.
Adapted from Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696–699.

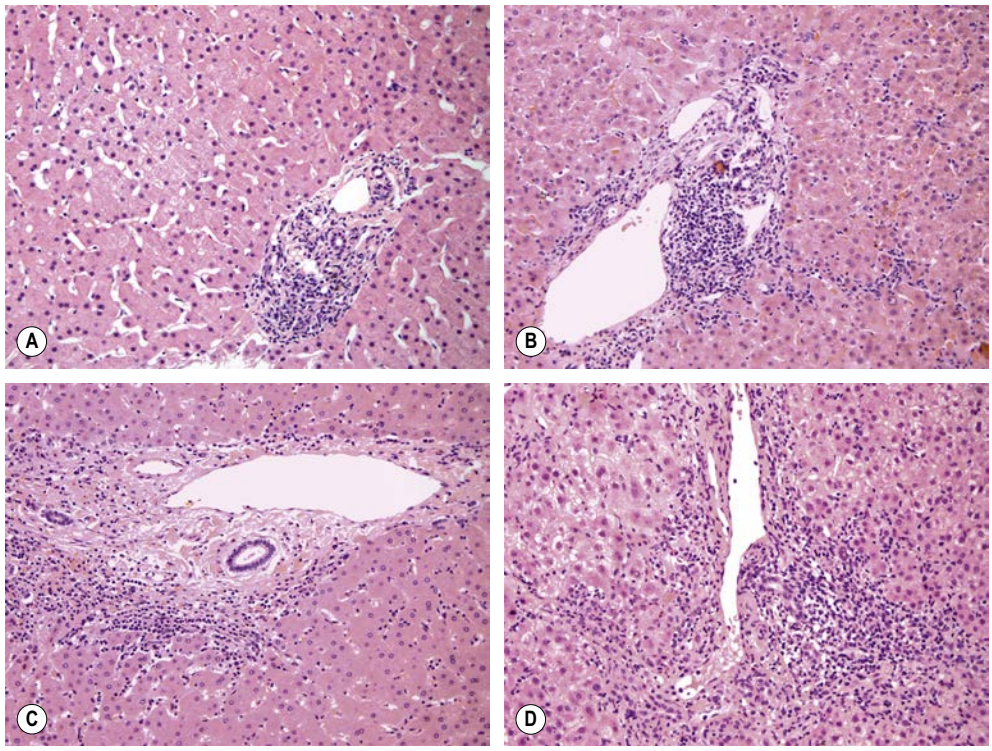

Fig. 9.21 Grading of chronic hepatitis. The examples of grading shown in these four panels emphasise the portal/periportal necroinflammatory component of chronic hepatitis; lobular activity also should be taken into account, but is frequently less prominent. **A:** Minimal activity (grade 1). Inflammation is confined to the portal tracts, and there is no interface hepatitis. The lobular parenchyma is quiescent. **B:** Mild activity (grade 2). Focal interface hepatitis is now present (right periportal region) in addition to portal tract inflammation. A few lobular necroinflammatory foci are also seen at right. **C:** Moderate activity (grade 3). More extensive interface hepatitis is present than in grade 2 but involving <50% of the circumference of most portal tracts. In this example, the portal tract edges above and to the right show relative sparing. **D:** Marked activity (grade 4). The portal tract is diffusely inflamed and shows extensive circumferential interface hepatitis. Similar changes affect virtually all portal tracts with this grade of activity, often with considerable lobular activity. (Needle biopsies, H&E.)

microscope. In a clinical trial, it may be helpful to reassess a proportion of biopsies in order to test intraobserver variation. The scoring should be performed by the same observer or observers throughout, and is usually done without knowledge of clinical data.

Interpretation of the results of scoring

When the scores from a group of biopsies are assessed, the statistical methods used to evaluate the results must be appropriate for categorical data. An example of a suitable method is that used by Lagging et al.[155] Some grading systems are divided into several categories. In the case of the Ishak score, these are interface hepatitis, confluent necrosis, lobular activity and portal inflammation. For each of these four categories the scale from 0 to 4 or 0 to 6 is not exactly linear, and it is therefore not acceptable to add the four scores together and then to manipulate the result as if it were a true mathematical sum. To put the matter another way, a score of 2 for any particular feature does not denote exactly twice 1 or precisely half of 4, but simply a score somewhere between 1 and 3. Nevertheless, total grading scores are often used

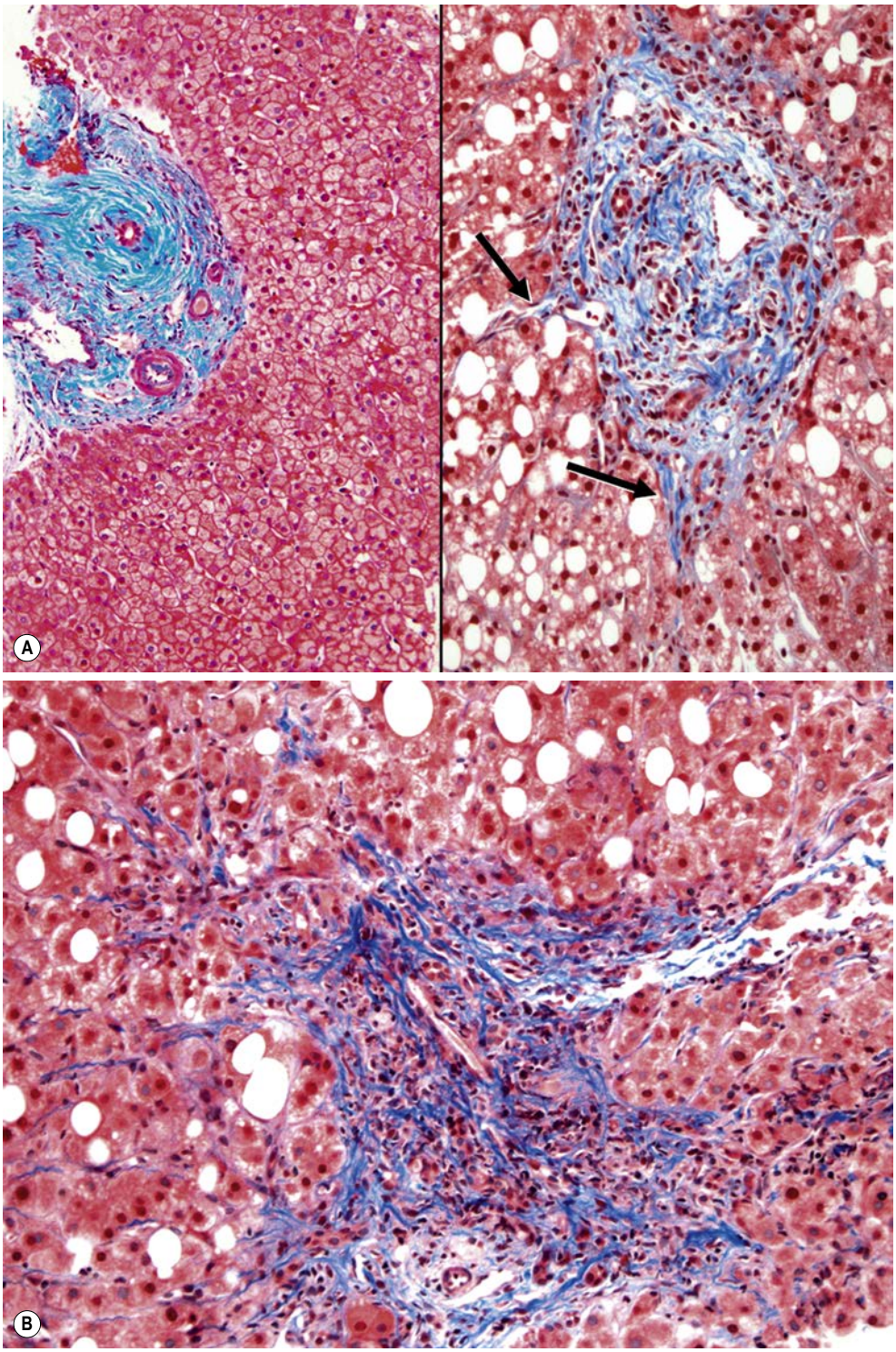

Fig. 9.22 Staging of chronic hepatitis. A: Minimal fibrosis (stage 1). This type of modest fibrosis sometimes takes the form of rounded fibrous expansion of some portal tracts (shown at left). In other cases, minimal fibrosis consists of occasional short fibrous scars at the edges of some, but not all, portal tracts (shown at arrows in right panel). **B:** Mild fibrosis (stage 2). Most portal tracts have a stellate contour due to periportal fibrosis, as shown here.

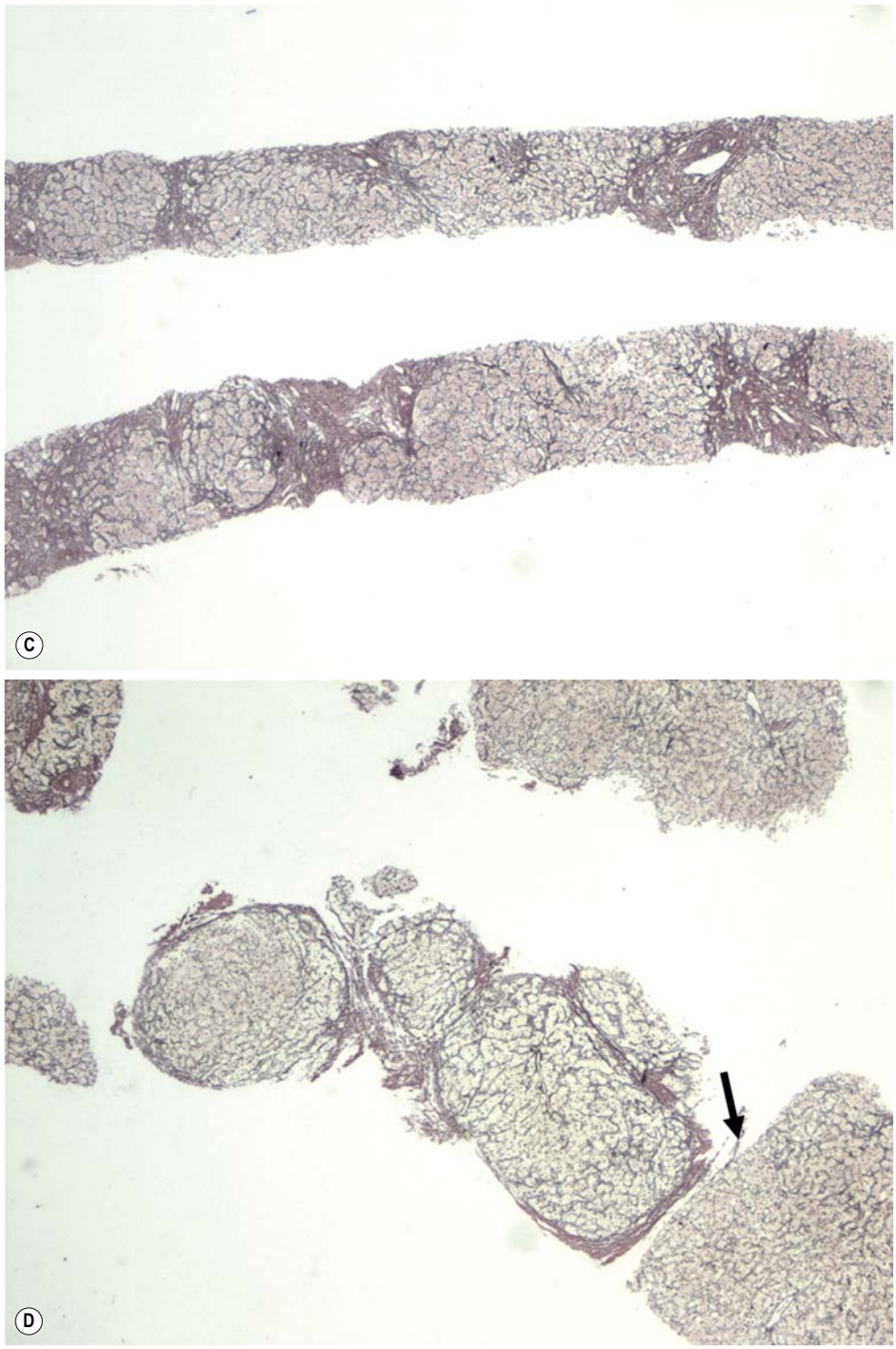

Fig. 9.22 cont'd
C: Extensive bridging fibrosis with nodularity but without cirrhosis (stage 3). This biopsy specimen consisted of several cores of liver tissue. Portal-to-portal bridging fibrosis is prominent in the left-hand portions of both cores, while more architecturally preserved parenchyma with stellate scarring of portal tracts is evident at right. Developing parenchymal nodules are also seen at left. Fully established cirrhosis is not demonstrated. D: Cirrhosis (stage 4). Architecturally abnormal regenerative nodules are evident and are circumscribed by diffuse fibrosis. Needle biopsy samples sometimes contain fragments of cirrhotic nodules (at arrow) which have been shelled out and separated from the adjacent fibrous septa. Such regenerative fragments frequently have a 'squared-off' or 'flat-top' appearance at their edges. (A and B, needle biopsies: trichrome stain; C and D, needle biopsies: reticulin stain.)

and published. In routine practice a total grading score gives an approximate indication of the severity of a patient's hepatitis but no information on the relative contribution of each category. In clinical trials of new therapies, total grading scores are potentially misleading, and the effect of the therapy on each individual grading category should be examined.

The possibility or indeed likelihood of sampling variation must also be kept in mind.[156] In chronic hepatitis C, there are differences between the findings in the left and the right lobe of the liver.[157] More importantly, small-needle biopsy samples may be misleading. Recent studies have thrown light on this particular issue. In one study using image analysis to assess fibrosis, the ability of the image analysis to predict a METAVIR fibrosis score diminished progressively in specimens less than 25 mm long.[148] In another study, reducing the sample size optically led to underestimation of disease severity in samples less than 20 mm long and 1.4 mm wide.[158] According to this study, samples obtained with fine needles were considered unsatisfactory for scoring. Another group recommended that the use of fine needles in diffuse HCV-related liver disease should be restricted to early non-fibrotic lesions.[159] While not all investigators agree that fine-needle specimens are inadequate for grading and staging,[160] specimen size is clearly a critical issue.

In conclusion, many factors limit the accuracy of semi-quantitative scoring in chronic hepatitis.[161] Awareness of these factors, careful attempts to minimise observer variation and appropriate interpretation of the results should ensure the continued usefulness of grading and staging in clinical practice and research.

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CHAPTER 10

Cirrhosis

Introduction

Cirrhosis is a diffuse process in which the normal lobules are replaced by architecturally abnormal nodules separated by fibrous tissue.[1,2] The nodules, which are most commonly the result of regenerative hyperplasia following hepatocellular injury, are functionally less efficient than normal hepatic parenchyma and there is a profound disturbance of vascular relationships.

Several different kinds of information can be obtained about the cirrhotic liver by means of liver biopsy (**Box 10.1**). The most important functions of biopsy are to establish a diagnosis, to assess the cause of the cirrhosis as far as possible and to detect hepatocellular carcinoma (HCC).

Box 10.1 Main information from liver biopsy in cirrhosis

Diagnosis of cirrhosis
Assessment of cause
Stage of development
Histological activity
Detection of hepatocellular carcinoma

Diagnosis of cirrhosis by liver biopsy

The ease with which the pathologist can diagnose cirrhosis from a biopsy specimen depends on the sample as well as on the criteria used. On the one hand, the sample may be sufficiently big, and the nodules sufficiently small, to make the diagnosis obvious. On the other hand, a slender core from within a large cirrhotic nodule can be difficult to identify as such (**see Fig. 1.6**). There are occasions when the pathologist can do no more than hint at the possible diagnosis.

The type of biopsy needle used also influences the ease of diagnosis. Very narrow needles may be adequate to obtain tumour samples, but may be inadequate for the accurate diagnosis of medical conditions. For example, in staging chronic hepatitis thin-needle biopsies obtained under computed tomographic guidance may underdiagnose cirrhosis for advanced bridging fibrosis.[3] Some clinicians prefer to use the TruCut type of needle when cirrhosis is suspected in order to lessen the risk of fragmentation,[4,5] but suitable samples can usually be obtained with needles of the aspiration type.[6,7] Transjugular biopsy is used when there is a risk of haemorrhage by other routes. The combination of biopsy with laparoscopy has been advocated.[8,9] Operative wedge biopsies of cirrhotic liver give an accurate idea of the relative proportions of parenchyma and stroma in the liver as a whole.[10]

The histological criteria for a diagnosis of cirrhosis are outlined in **Box 10.2**. The two fundamental criteria, nodularity and fibrosis, reflect the definition of cirrhosis. When there are well-defined, rounded nodules surrounded by fibrous septa, the diagnosis is easily established. Underestimating the stage of fibrosis because of specimen fragmentation (see later) is a concern, particularly when scoring biopsies in chronic hepatitis.[11] Correlation with clinical and laboratory data helps surmount this problem. Occasionally,

Box 10.2 Cirrhosis: diagnostic criteria

Fundamental
Nodularity
Fibrosis
Relative
Fragmentation
Abnormal structure
Hepatocellular changes
Regenerative hyperplasia
Pleomorphism
Large-cell dysplasia (large-cell change)
Small-cell dysplasia (small-cell change)
Excess copper-associated protein

a nodular appearance just deep to the liver capsule is not representative of the whole liver but has resulted from transection of a tongue or peninsula extending from the main bulk of the parenchyma.

In many patients the relative criteria listed in **Box 10.2** are equally important. They allow a tentative diagnosis of cirrhosis to be reached, readily converted to a firm diagnosis when correlated with other data. A diagnosis of cirrhosis therefore requires communication between pathologist and clinician, and cannot be exactly equated with a histological stage.[12]

Fragmentation

Fragmentation of the specimen, either at the time of biopsy or during processing in the laboratory, should itself suggest the possibility of cirrhosis (**Fig. 10.1**). The specimen is more likely to break into fragments when needles of the aspiration type (e.g. Menghini) are used. Other biopsy specimens that are likely to fragment are metastatic tumours surrounded by reactive fibrous tissue, and HCC.

Abnormal structure

Structural changes should be assessed by means of a reticulin preparation, preferably not counterstained. This may show two features not readily seen with other stains. First, although nodules are readily cored out of the dense fibrous stroma of a cirrhotic liver during aspiration biopsy, a thin layer of connective tissue tends to adhere to the nodules over much of their surface (**Fig. 10.2**). This layer may be difficult to see even with the help of collagen stains, and is easily missed in haematoxylin and eosin (H&E)-stained sections (**Fig. 10.3**). Second, minor alterations of structure become apparent even in those nodules which

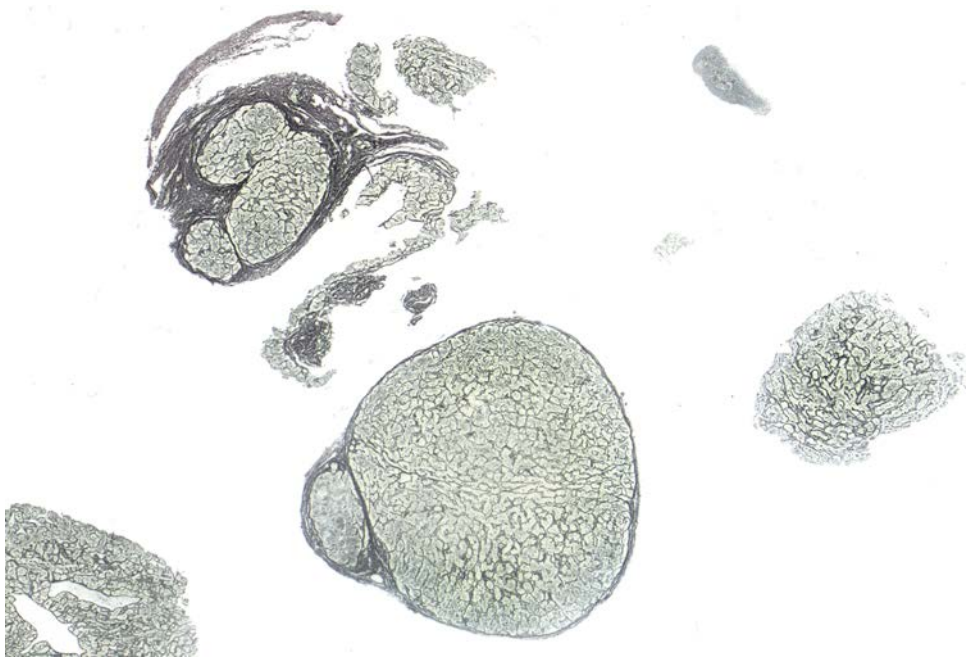

Fig. 10.1 Cirrhosis: fragmented sample. A specimen obtained by the aspiration biopsy method has broken into rounded fragments peripherally circumscribed by fibrosis. (Needle biopsy, reticulin.)

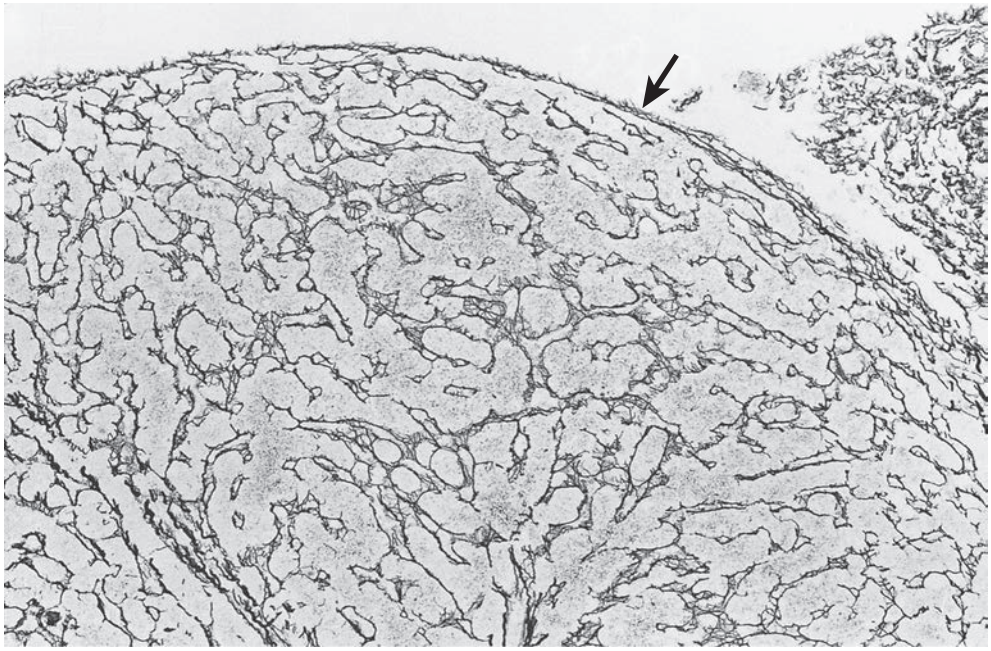

Fig. 10.2 Cirrhosis: selective sampling. A nodule has been cored out of the connective tissue by the biopsy procedure, but a thin layer of connective tissue (arrow) has adhered to the nodule margin. (Needle biopsy, reticulin.)

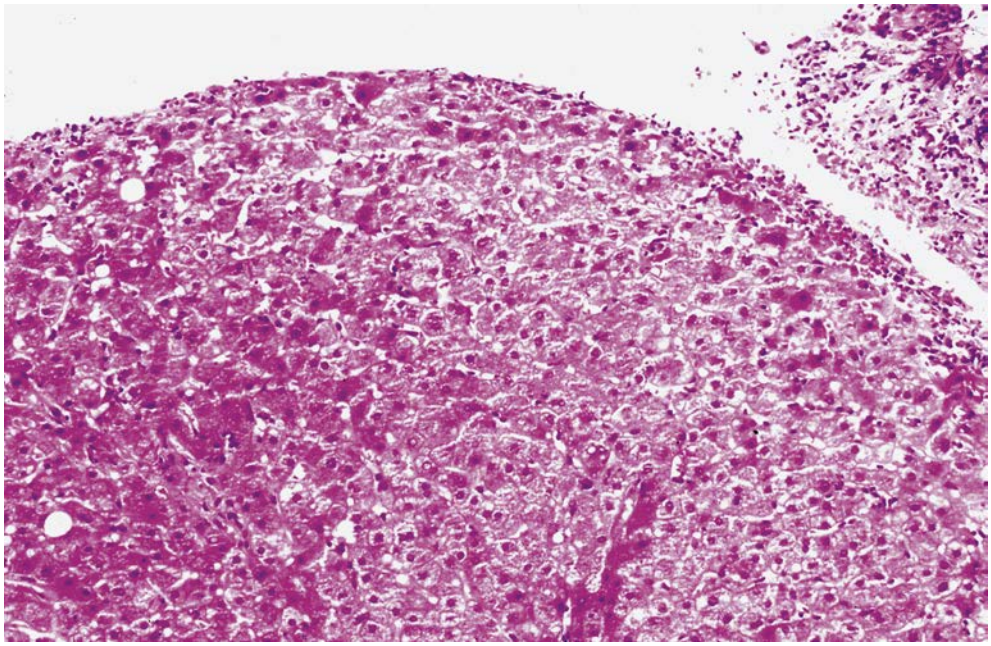

Fig. 10.3 Cirrhosis: selective sampling. Same field as in Fig. 10.2. In a haematoxylin and eosin preparation the thin layer of connective tissue is not easily seen. (Needle biopsy, H&E.)

closely mimic normal liver. Such alterations include abnormal orientation of reticulin fibres resulting from different patterns and rates of growth in different areas (**Fig. 10.4**) and approximation of portal tracts and terminal venules. The number of venules may be abnormally large in relation to the number of portal tracts (**Fig. 10.5**), and the latter are sometimes abnormally small and poorly formed (**see Fig. 1.4**). A more obvious structural abnormality in cirrhosis is the presence of septa linking central veins (terminal hepatic venules) to portal tracts. These septa must be distinguished from recently formed necrotic bridges.

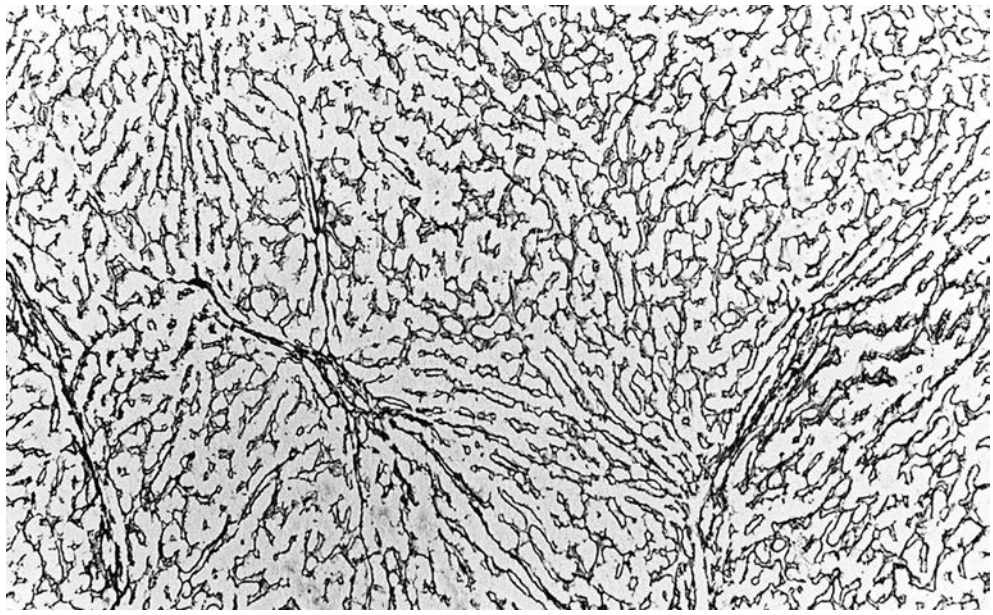

Fig. 10.4 Cirrhosis: distorted reticulin pattern. The distortion has resulted from abnormal and irregular hepatocyte growth patterns. (Needle biopsy, reticulin.)

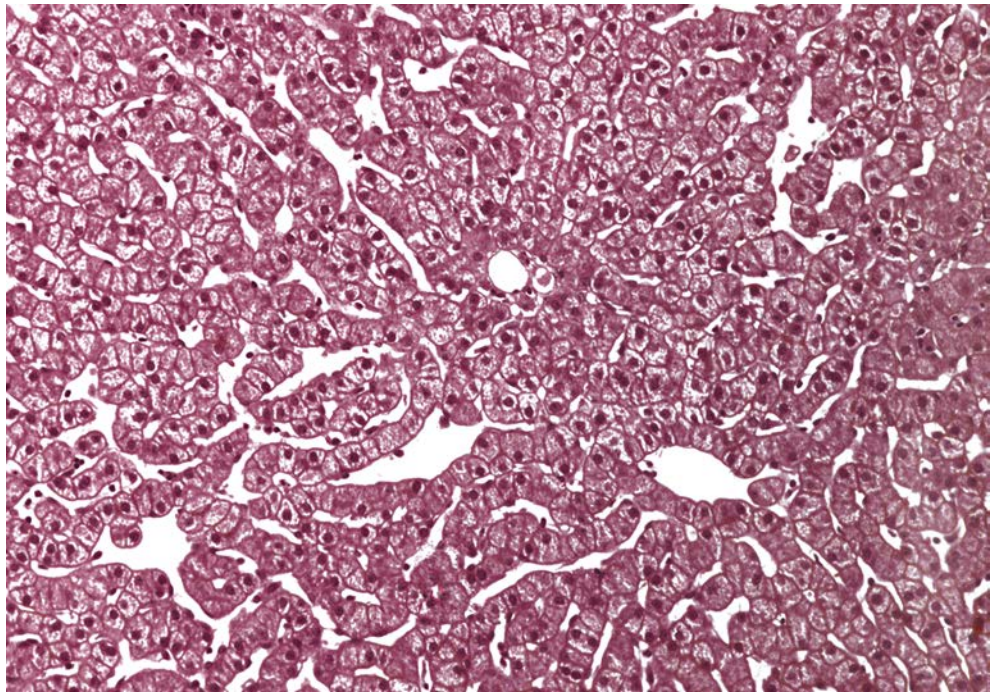

Fig. 10.5 Cirrhosis: abnormal vascular relationships. Several venous channels are seen near to each other. (Wedge biopsy, H&E.)

In wedge biopsies, excess fibrous tissue in and near the capsule and crowding of vessels must be distinguished from the changes of cirrhosis. The latter extend through the specimen, whereas the former is confined to the capsular and immediately subcapsular area.[13] Very occasionally a wedge biopsy of part of a large, well-differentiated regeneration nodule fails to show the histological features of cirrhosis.

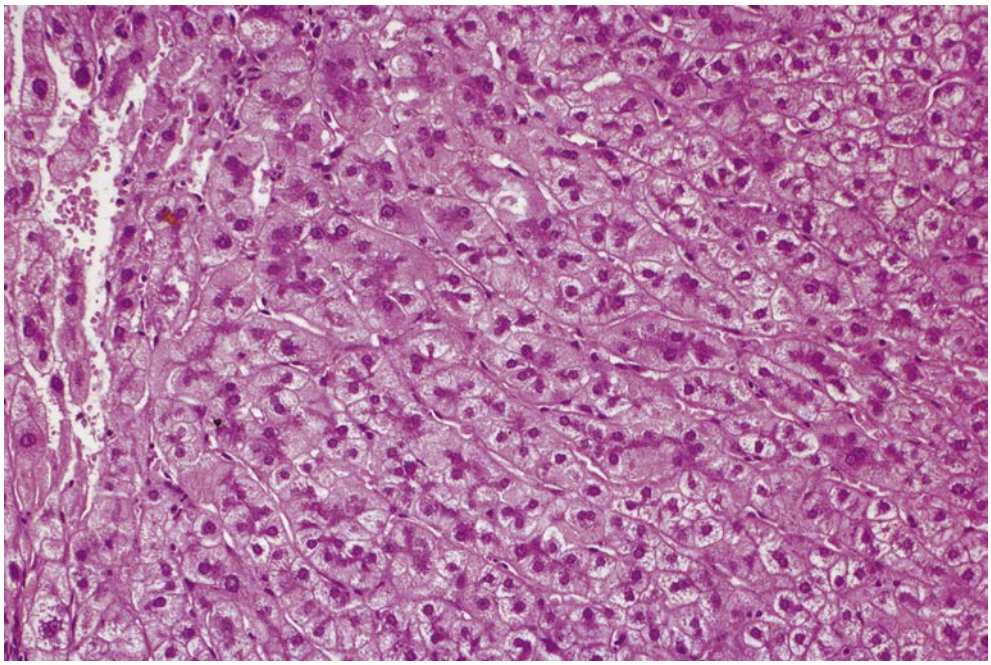

Fig. 10.6 Cirrhosis: hepatocellular regeneration. Liver-cell plates are two or more cells thick, indicating active growth. (Needle biopsy, H&E.)

Hepatocellular changes

In some biopsies from cirrhotic livers the hepatocytes are normal in appearance and arrangement, so that diagnosis rests on the structural changes discussed earlier. In others there are more or less obvious abnormalities of growth.

Regeneration is suggested by thickening of the liver-cell plates (**Fig. 10.6**). In any liver an oblique plane of sectioning will cause a few plates to appear more than one cell thick, but widespread double-cell plates are seen when there is active growth. Hepatocytes in hyperplastic areas contain little or no lipofuscin pigment, even near terminal venules. Regeneration is not always evident in cirrhosis because it is not a continuous process. Its absence does not therefore exclude the diagnosis. Conversely, its presence does not prove cirrhosis because it is found also in other circumstances, for example after an acute hepatitis and in the precirrhotic stages of chronic biliary diseases.

A very characteristic feature of cirrhosis is the presence of adjacent populations of hepatocytes growing at different rates and having different cell and nuclear characteristics (**Fig. 10.7**). This **pleomorphism** gives rise to the abnormalities of reticulin pattern already mentioned, notably a tendency for reticulin fibres in the different growth areas to lie in different directions.

In a minority of cirrhotic livers the hepatocytes show structural atypia of a degree sufficient to warrant a label of **dysplasia**, an appearance further discussed in **Chapter 11**. Two types have been described: large-cell dysplasia[14] and small-cell dysplasia.[15] Because of the controversial status of either type as a precursor of malignant change,[16] some authors prefer to call them large-cell change and small-cell change.[16,17] In the large-cell form, the cells are enlarged and their nuclei are hyperchromatic and irregular in shape, with prominent nucleoli (**Fig. 10.8**). Nuclear–cytoplasmic ratio is normal or only moderately increased.[18] This type of dysplasia was first described in an African population with a high incidence of HCC and hepatitis B virus (HBV) infection.[14] It is most often seen in patients with HBV and HCV infection but may also be evident in other chronic liver diseases.[19] There is evidence of an association of large-cell dysplasia with an increased risk of development of HCC independently of other risk factors.[20,21] Decreased expression of cell cycle checkpoint

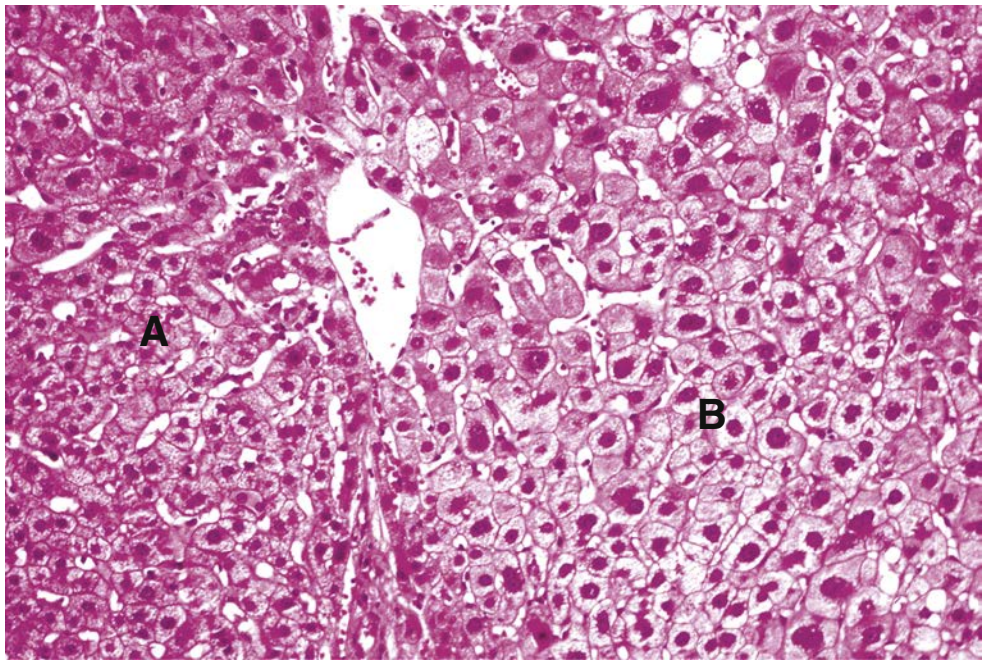

Fig. 10.7 Cirrhosis: different cell populations. The parenchymal cells in area A are smaller than those in area B, which also show a rounded and nodular growth pattern. (Wedge biopsy, H&E.)

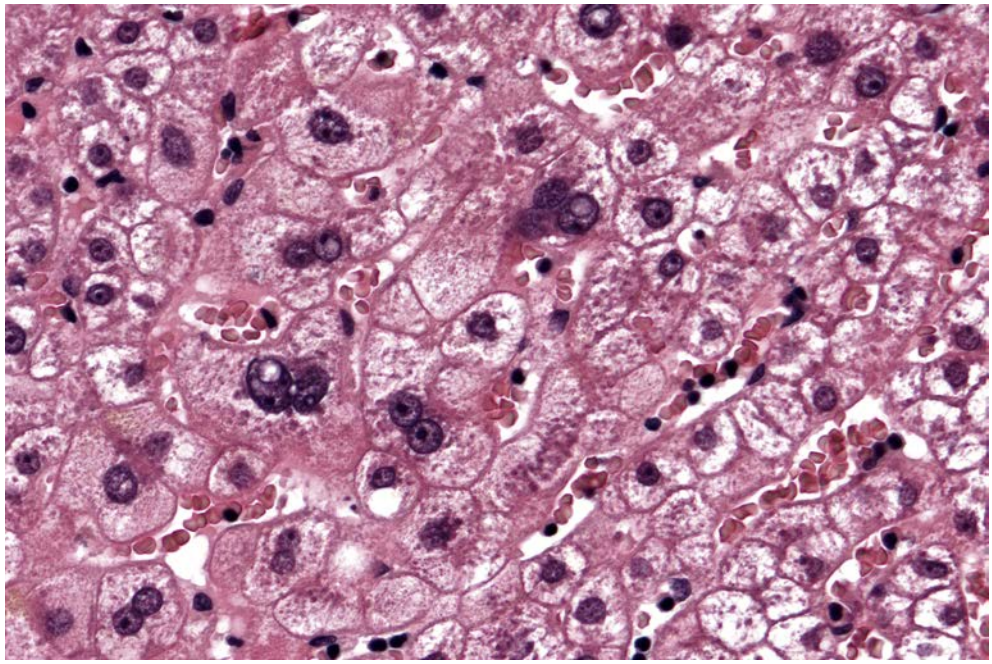

Fig. 10.8 Cirrhosis: large-cell dysplasia (large-cell change). The nuclei of the enlarged hepatocytes at centre and left are irregular in shape and vary greatly in size and staining intensity. Several of these cells are multinucleated. Compare with the normal hepatocytes at right and in the upper left-hand corner. (Wedge biopsy, H&E.)

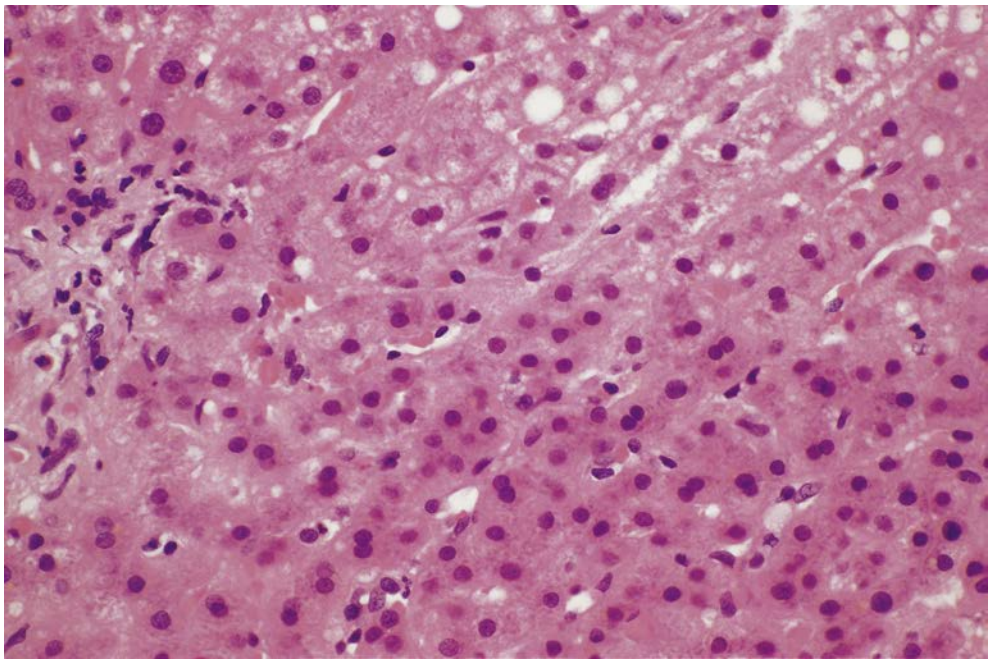

Fig. 10.9 Cirrhosis: small-cell dysplasia (small-cell change). The hepatocytes below and to the right have normal-sized nuclei, but their overall size is reduced. Nuclear–cytoplasmic ratios are therefore increased. (Needle biopsy, H&E.)

markers, presence of cytoplasmic DNA micronuclei and shortened telomeres in large-cell change are evidence favouring a disposition to HCC.[22] Demonstration of an increased hepatocyte proliferation rate as a risk for carcinoma is also important.[23] Care should be taken not to interpret the nuclear atypia which may be associated with cholestasis as large-cell dysplasia.[17]

In small-cell dysplasia the nuclear–cytoplasmic ratio is increased but the overall size of the affected cells is less than normal (**Fig. 10.9**). Zones of dysplastic hepatocytes of either type support a diagnosis of cirrhosis and are regarded by some clinicians as an indication for increased monitoring for HCC. A finding of dysplasia of either type should therefore be specifically mentioned in liver biopsy reports.

Differential diagnosis

When there is nodularity and evidence of regeneration but little or no fibrosis, **nodular regenerative hyperplasia** should be considered. In **congenital hepatic fibrosis** the acinar architecture remains intact and the ductal plate malformation is seen. In **chronic hepatitis** with fibrosis and structural abnormalities, the differential diagnosis is between active cirrhosis and chronic hepatitis that has not yet reached the stage of cirrhosis. This problem cannot always be resolved on the basis of a liver biopsy. Similar doubt may arise in steatohepatitis. The presence of substantial quantities of copper and copper-associated protein in non-cholestatic chronic liver disease supports a diagnosis of cirrhosis.[24] Cirrhotic nodules can usually be distinguished from well-differentiated **HCC**. In the latter the cell plate architecture is more abnormal, reticulin may be scanty or absent and the cells have malignant cytological characteristics. Also, hepatocellular siderosis is often present secondarily in cirrhosis of varied aetiology (**Ch. 14**) but is typically absent in tumour cells of HCC.

Assessment of cause

Box 10.3 Main causes of cirrhosis
Viral hepatitis (B, C, D)
Alcohol abuse
Obesity, insulin resistance/metabolic syndrome
Biliary disease
Metabolic disorders
Haemochromatosis
Wilson's disease
α1-Antitrypsin deficiency, etc.
Venous outflow obstruction
Drugs and toxins
Autoimmune disease

Box 10.4 Cirrhosis: assessment of cause
Pattern of nodules and fibrosis
Bile ducts
Blood vessels
Steatohepatitis
Evidence of viral infection
Abnormal deposits
Iron
Copper, copper-associated protein
α1-Antitrypsin globules

Biopsy may help to establish the cause of a cirrhosis. In some of the categories listed in **Box 10.3** the histological appearances are diagnostic. The term 'cryptogenic' should only be applied when full clinical and laboratory investigations have been completed and the features listed in **Box 10.4** have been assessed. This can be achieved by means of a small range of routine stains. There is evidence to suggest that many examples of cryptogenic cirrhosis result from non-alcoholic steatohepatitis (NASH), not evident histologically at the time of diagnosis.[25] Some cases of cirrhosis are due to mutations in genes for specific cellular keratins[26] or for bile canalicular transporter proteins.[27]

Pattern of nodules and fibrosis

Irregularly shaped nodules suggest the possibility of a biliary cause, especially if there is perinodular oedema, ductular reaction and chronic cholestasis. In a precirrhotic stage of venous outflow obstruction there is regular fibrosis in perivenular regions (acinar zones 3). Sinusoids are dilated. Portal tracts show little or no abnormality or sometimes have changes mimicking biliary tract obstruction.[28] Persistence of spared portal tracts when late, progressive perivenular fibrosis and cirrhosis have developed often results in the appearance of 'reversed lobulation' (**Fig. 10.10**) with a relatively normal portal tract (rather than a central vein) now present at the centre of the parenchymal unit. Chronic hepatic venous outflow obstruction (e.g. chronic Budd–Chiari syndrome, chronic congestive hepatopathy of cardiac failure) and late, inactive steatohepatitis are exponents of 'reversed lobulation'.

Certain features are indicative of earlier chronic hepatitis that evolved to cirrhosis. Irregular, slender fibrous septa emanating from portal tracts, lymphoplasmacytic infiltrates, lymphoid aggregates or follicles and foci of interface hepatitis should prompt consideration of the several causes of chronic hepatitis.

Confluent fibrosis which replaces multiple adjacent lobules is a common feature in several types of cirrhosis, particularly following steatohepatitis and chronic hepatitis of viral or autoimmune aetiology. It is also seen in the less common 'postnecrotic' cirrhosis which develops rapidly, within a few months of a severe viral or drug-induced acute hepatitis. Needle biopsy samples in such cases may show entire cores, portions of cores and especially the subcapsular region occupied by fibrous tissue, residual portal tracts and many ductular structures (**see Fig. 4.13C**), mild chronic inflammatory cell infiltrates, entrapped regenerative liver-cell rosettes and collections of small neovessels (see the 'Blood vessels' section).

Bile ducts

Assessment of bile-duct numbers in cirrhosis is very important. The number of ducts should approximately equal the number of arteries of similar size and location, but the pathologist must bear in mind that not every portal tract will necessarily contain a bile duct in the plane of section. Definite duct loss should prompt consideration of primary

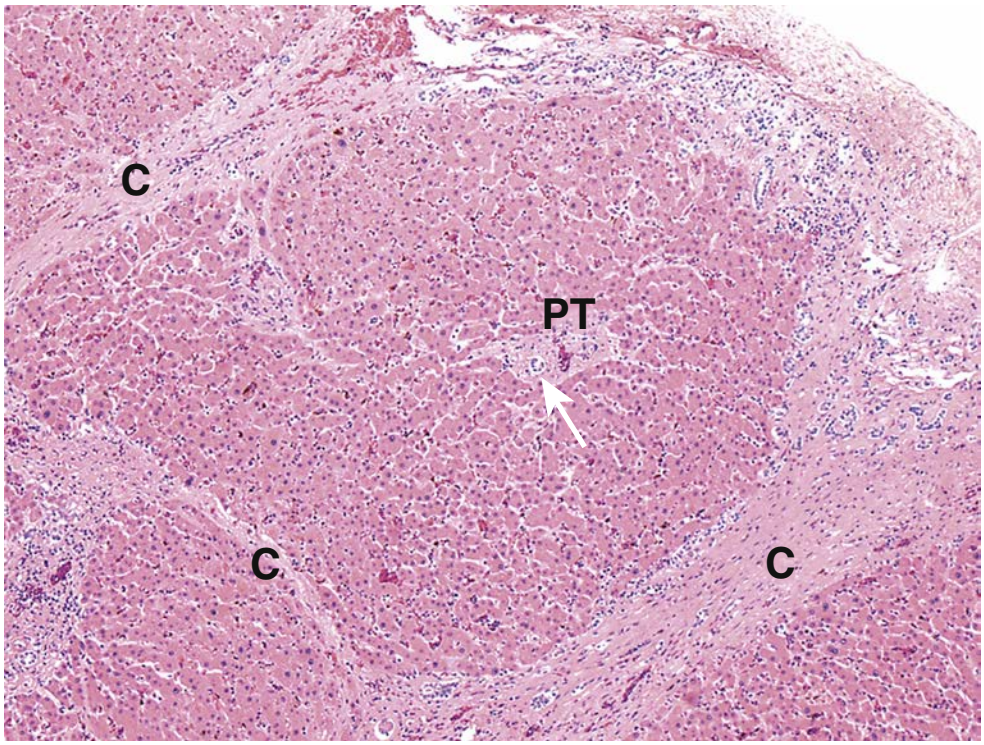

Fig. 10.10 'Reversed lobulation' in late cardiac cirrhosis. A portal tract (PT) with visible bile duct (arrow) is at the centre of the regenerative nodule in this cirrhosis which followed decades of biventricular cardiac failure (congestive hepatopathy). Chronic damage to and fibrosis of centrilobular regions (C) as is seen with long-standing cardiac disease often result in central-to-central bridging fibrosis, leaving spared portal tracts at the centres of regenerative nodules (arrow), a pattern known as 'reversed lobulation'. (Postmortem liver, H&E.)

biliary cirrhosis or primary sclerosing cholangitis. In some cases ductopenia is drug-related or is associated with other conditions,[29] so the clinical history is important. In children or young adults, other ductopenic syndromes should also be considered. Typical bile-duct lesions of primary biliary cirrhosis, with or without granulomas, are still sometimes found at a stage of cirrhosis.

Periductal fibrosis may be very prominent in primary sclerosing cholangitis. Ductular reaction is a non-specific finding, but when severe and focal, it often reflects biliary disease. Following extensive hepatocellular damage in cirrhosis—for example, after variceal haemorrhage—there is sometimes a very extensive ductular reaction which can be mistaken for cholangiocarcinoma.

Blood vessels

Occluded, narrowed or recanalised veins suggest that the cirrhosis may be the result of venous outflow block, but they are also found in cirrhosis from other causes.[30,31] Portal and hepatic venous thrombosis has indeed been implicated in the progression of cirrhosis in general.[32] Recognition of venous lesions is often difficult without the help of stains for collagen or elastic fibres. Neovascularisation of fibrotic portal tracts, areas of confluent fibrosis and bridging fibrous septa in cirrhosis produce numerous lymphatic and capillary channels, particularly in chronic hepatitis B and C.[33]

Steatohepatitis

This is found in alcohol abusers and in individuals at risk for non-alcoholic fatty liver disease, as a manifestation of drug toxicity, or for no obvious underlying reason (Ch. 7).

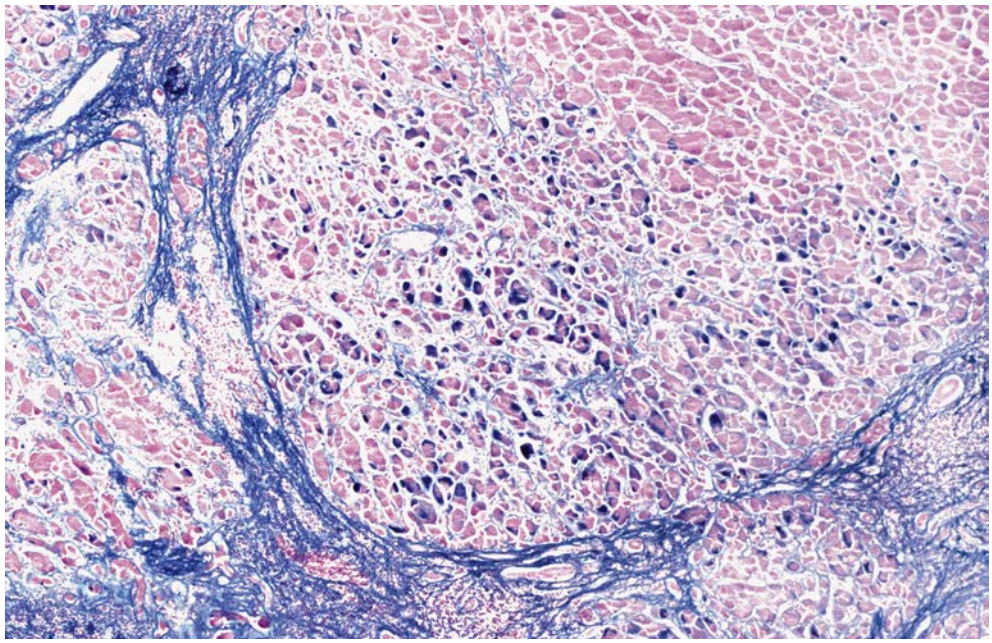

Fig. 10.11 Hepatitis B surface antigen in hepatitis B virus (HBV)-related cirrhosis. Many hepatocytes show positive cytoplasmic staining for HBV surface antigen. This staining method also demonstrates elastic tissue fibres in the fibrous tissue. (Recipient liver from transplantation, Victoria blue.)

In amiodarone toxicity the fatty change is usually absent. Steatohepatitis must be distinguished from chronic cholestasis, in which there are also swollen hepatocytes containing Mallory bodies (Ch. 5).

Evidence of viral infection

Features of chronic hepatitis, particularly interface hepatitis and lymphocytic infiltration, are often but by no means always due to infection with one of the hepatitis viruses. Liver-cell dysplasia also favours a viral cause. Ground-glass hepatocytes, Victoria blue or orcein stains (Fig. 10.11) and immunostains for viral antigens (**see Fig. 9.14**) help in the diagnosis of HBV infection, but tissue evidence of HBV antigens is not always present or detectable. Lymphoid aggregates or follicles should suggest the possibility of hepatitis C (Fig. 10.12). More than one virus or other causal agent may be responsible for a patient's cirrhosis. Abundant plasma cells raise the possibility of autoimmune hepatitis but are also sometimes found in viral hepatitis.

Abnormal deposits

Severe parenchymal siderosis should always raise the possibility of hereditary haemochromatosis, even when another cause is also evident. However, stainable iron often accumulates in cirrhosis from any cause.[34,35] Lack of significant haemosiderin in the connective tissue of portal tracts or fibrous septa in a cirrhosis points to a cause other than hereditary haemochromatosis (**see Fig. 14.13**). In hereditary haemochromatosis the nodules are sometimes irregular, as in biliary cirrhosis.

Copper and copper-associated protein can often be detected in cirrhosis, whatever its cause.[24] Large amounts at the edges of the nodules suggest biliary disease. Staining of entire nodules is seen in Wilson's disease, but other nodules may be negative. In some stages of the disease the copper is not histochemically demonstrable, so that negative staining does

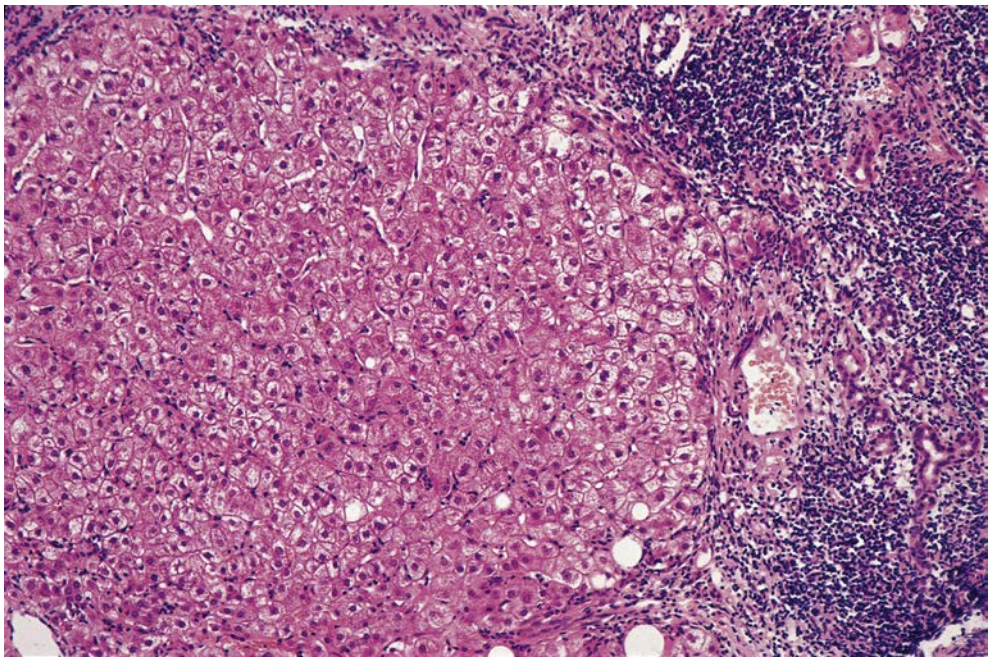

Fig. 10.12 Cirrhosis following hepatitis C virus infection. Lymphoid aggregates are still visible. The patient was also infected with GBV-C (the so-called hepatitis G virus). (Recipient liver from transplantation, H&E.)

not exclude the diagnosis. Abundant copper and Mallory bodies are also features of Indian childhood cirrhosis and other forms of copper toxicosis.[36,37]

Alpha$_1$-antitrypsin bodies should always be looked for in cirrhosis. Immunocytochemical staining is more sensitive than diastase–periodic acid–Schiff.

Anatomical type

Because of possible sampling error, the pathologist cannot confidently assess nodule size in the rest of the liver on the basis of a biopsy specimen. This is usually of little consequence to the patient, though one biopsy study has suggested a significant correlation of the hepatic venous pressure gradient in portal hypertension with small nodule size.[38] Nevertheless, primary classification of cirrhosis by nodule size is no longer appropriate, because aetiology is clinically much more important.

However, nodule size does influence the ease of histological diagnosis. When nodules are of the size order of the lobules from which they are derived, several nodules are usually seen in one biopsy and diagnosis is easy (**Figs 10.13 and 10.14**). When nodules are larger (**Fig. 10.15**), more subtle diagnostic criteria need to be considered. The most difficult anatomical type to recognise is **incomplete septal cirrhosis**. This is characterised by indistinct nodularity, slender septa, some of which end blindly, poorly formed small portal tracts and abnormal relationships between portal tracts and efferent venules[39] (**Fig. 10.16**). There is evidence of hepatocytic hyperplasia, giving rise to crowding of reticulin fibres in adjacent areas. Sinusoidal dilatation is common, while inflammation and necrosis are generally modest or absent. A reticulin preparation is important for diagnosis because the slender septa are easily missed (**Figs 10.16 and 10.17**). The diagnosis is more easily made in wedge biopsies than in needle specimens. A relationship to various forms of non-cirrhotic portal hypertension has been demonstrated,[39–42] but it has also been postulated that incomplete septal cirrhosis can represent a burnt-out form of macronodular cirrhosis.[43] The incomplete septa could also reflect resorption of fibrous tissue, a type of 'regressed cirrhosis'.

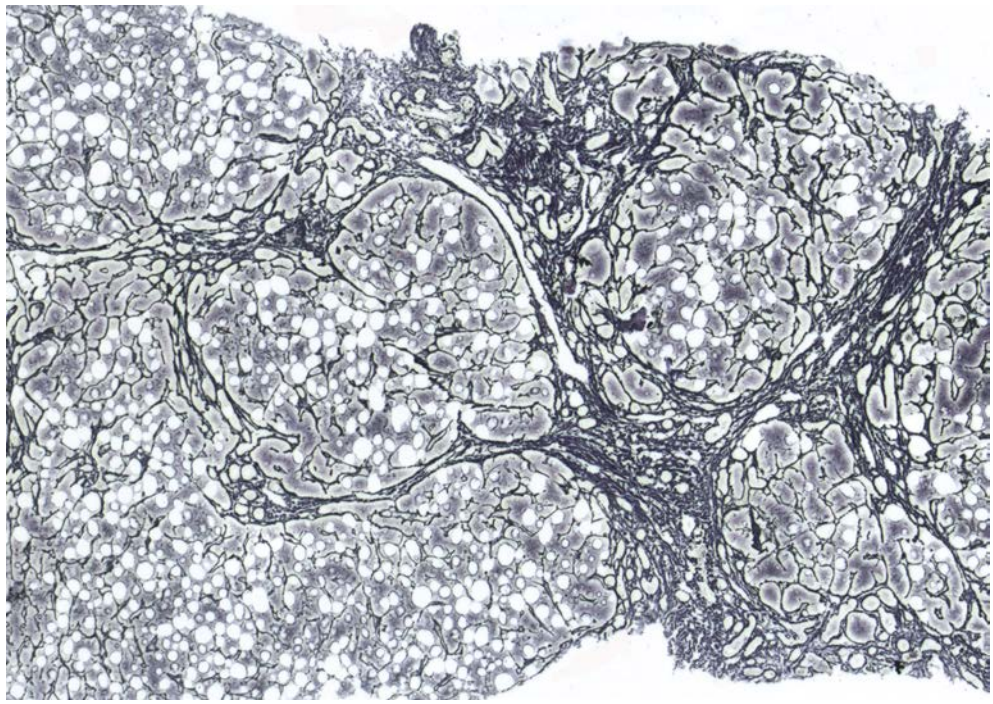

Fig. 10.13 Cirrhosis: micronodular pattern. Nodules are of lobular size or smaller. (Needle biopsy, reticulin.)

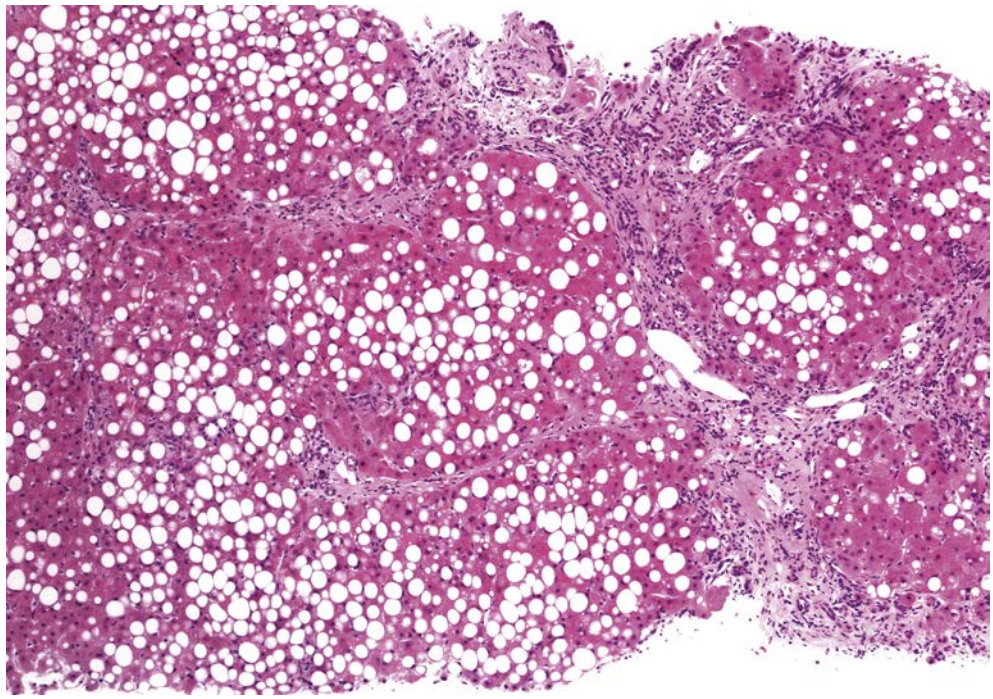

Fig. 10.14 Cirrhosis: micronodular pattern. Similar field as in Fig. 10.12. There is steatosis. (Needle biopsy, H&E.)

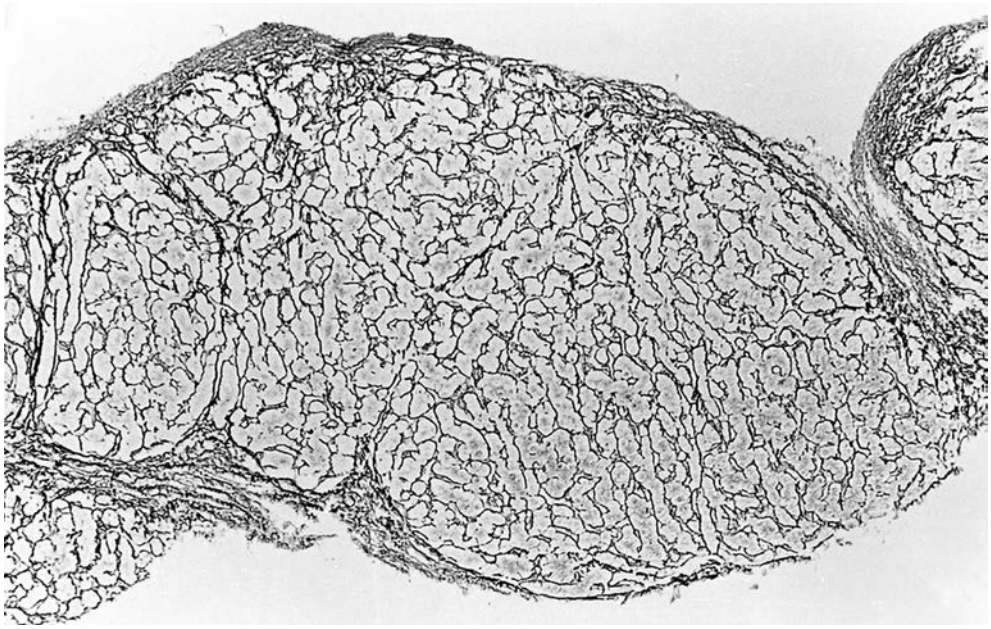

Fig. 10.15 Cirrhosis: macronodular pattern. Nodules are larger than in **Figs 10.12** and **10.13**. The magnification is slightly smaller. (Needle biopsy, reticulin.)

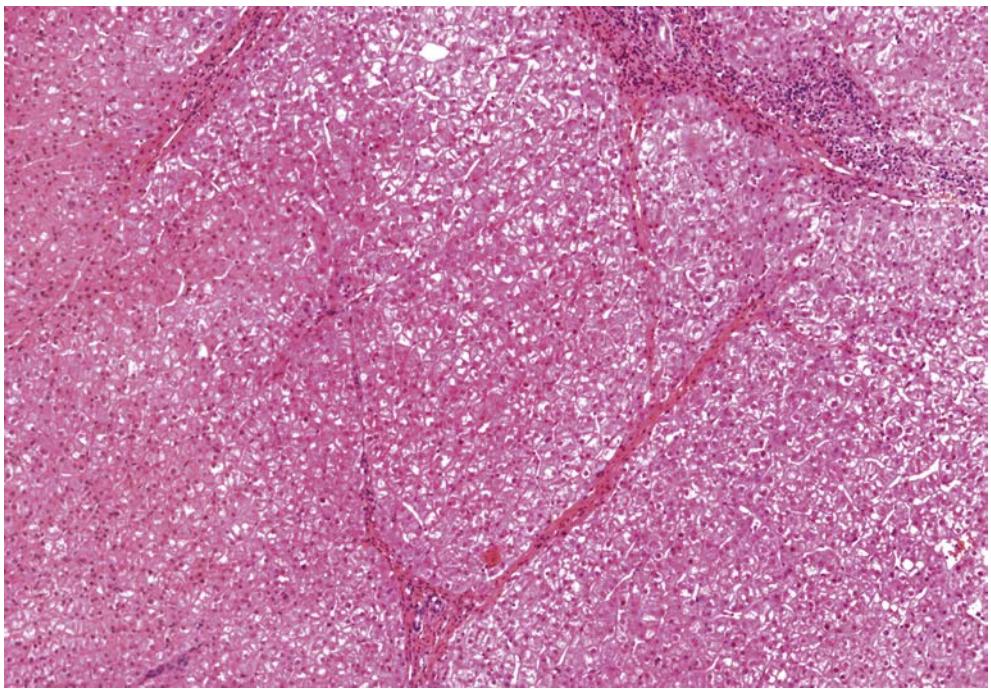

Fig. 10.16 Cirrhosis: incomplete septal pattern. The parenchyma is nodular but only partially surrounded by fibrous septa. Note the incomplete fibrous septum emerging vertically from the portal tract at bottom. (Wedge biopsy, H&E.)

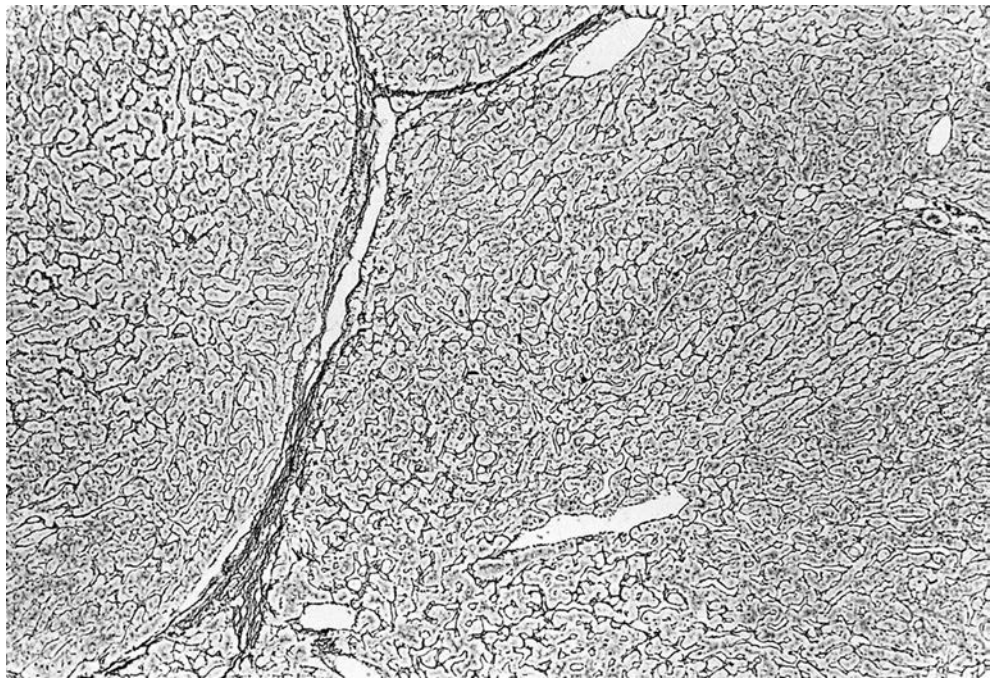

Fig. 10.17 Cirrhosis: incomplete septal pattern. Slender septa and vessels are present. There is a small portal tract on the right, towards the top of the figure. (Wedge biopsy, reticulin.)

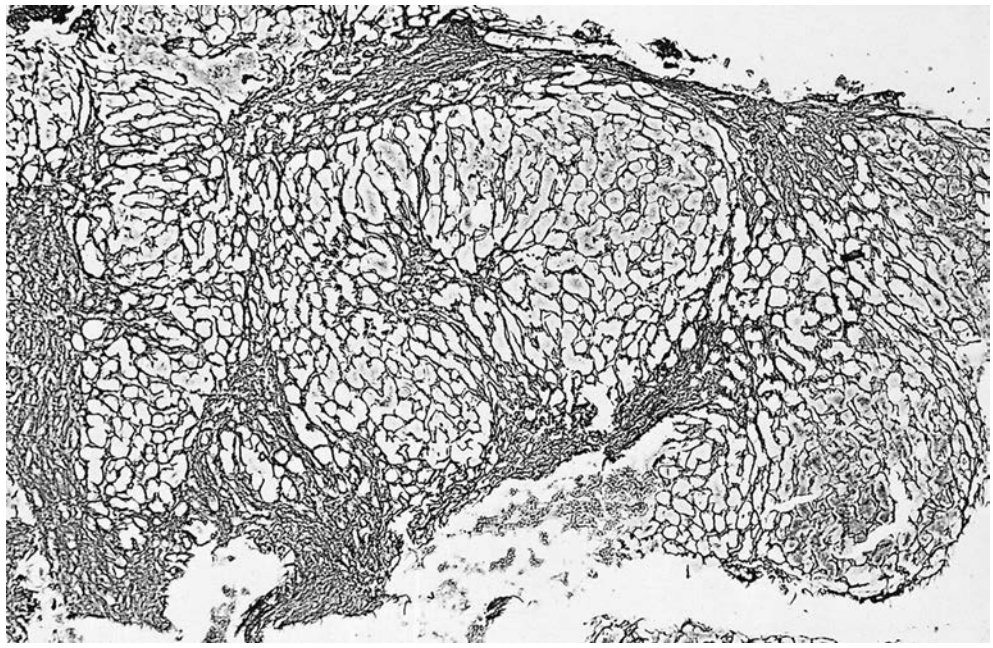

Fig. 10.18 Early (developing) cirrhosis. There is extensive fibrosis and architectural distortion in this biopsy from an alcohol abuser. Nodules are beginning to form but are not yet clearly defined. (Needle biopsy, reticulin.)

Stage of development

In some patients, cirrhosis is obvious and appears mature, in the sense that the well-demarcated nodules and dense fibrosis give an impression of long-standing disease. In others there is doubt as to whether there is cirrhosis or merely fibrosis. An impression may be gained that cirrhosis is incipient or at an early stage of development (Fig. 10.18). When

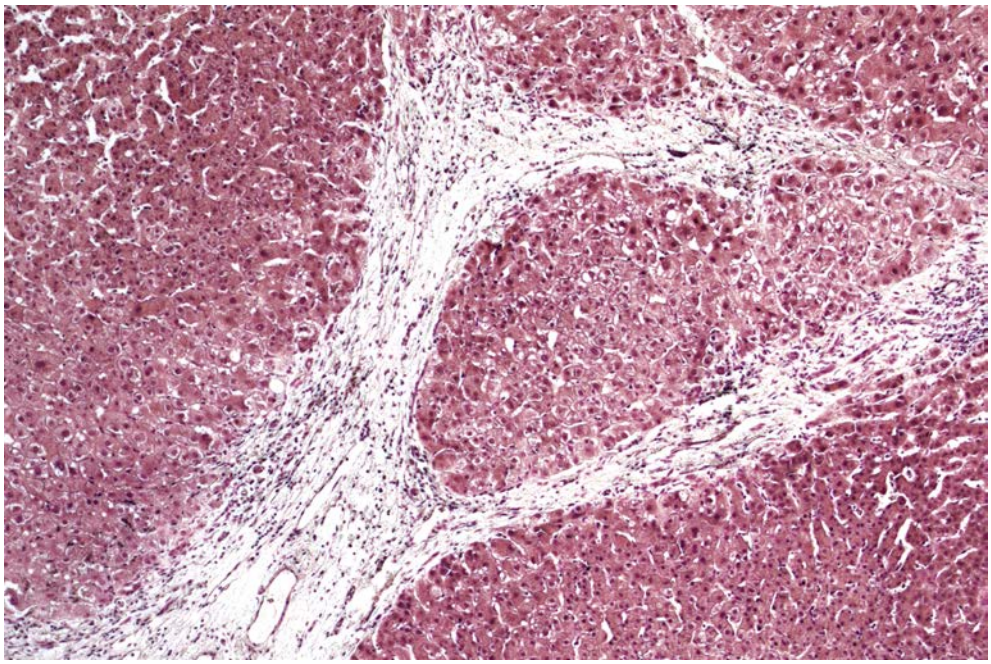

Fig. 10.19 Inactive cirrhosis. Nodules are sharply outlined and inflammatory cells are scanty. (Wedge biopsy, H&E.)

doubt is unresolved, a report of 'developing cirrhosis' or 'incomplete cirrhosis' is sometimes appropriate. The concept of incomplete cirrhosis is recognised in the staging system of Ishak et al.[44] Once cirrhosis is fully established, reversion to a normal lobular pattern has historically been considered unlikely. Reversibility of cirrhosis is a controversial subject,[45] and the pathologist should consider this prospect cautiously, taking into account the type of biopsy sample, the underlying disease process and the possibility of sampling error. The aetiology of the cirrhosis is influential on its potential for remodelling and/or regression[46] and this should be factored into evaluation of individual cases in the context of relevant clinical data. Diminished fibrosis following therapy does not automatically confer a return to normal liver-cell plate structure and vascular relationships.[2] Despite these caveats, reports of regression of cirrhosis of varied aetiologies[46-48] deserve attention.

Paradoxically, there may be confusion between mild chronic hepatitis and an inactive, well-established cirrhosis. This reflects difficulty in diagnosing some examples of late cirrhosis by needle biopsy because of a tendency for nodule size to increase with time.

Histological activity

Activity is a convenient term to describe the rate of progression of the cirrhosis. It is usually taken to mean the various forms of liver-cell damage and inflammation typical of chronic viral hepatitis. In cirrhosis following steatohepatitis, however, the severity of the latter should also be taken into account.

In an inactive cirrhosis the interface between septa and nodules is sharply defined (**Fig. 10.19**). Cellular infiltration is mild and may be confined to the septa. There is little or no focal necrosis or intranodular inflammation. In an active, rapidly progressive cirrhosis, by contrast, the interface is blurred by hepatocellular damage and inflammation (**Fig. 10.20**). Isolated hepatocytes or groups of cells may be seen within the inflamed septa. There is hepatocellular damage and inflammation deep within the nodules.

Histological activity often varies in severity from one part of the liver to another. Comparison of activity in multiple biopsies from an individual patient should therefore be made with caution and with reference to clinical and biochemical data.

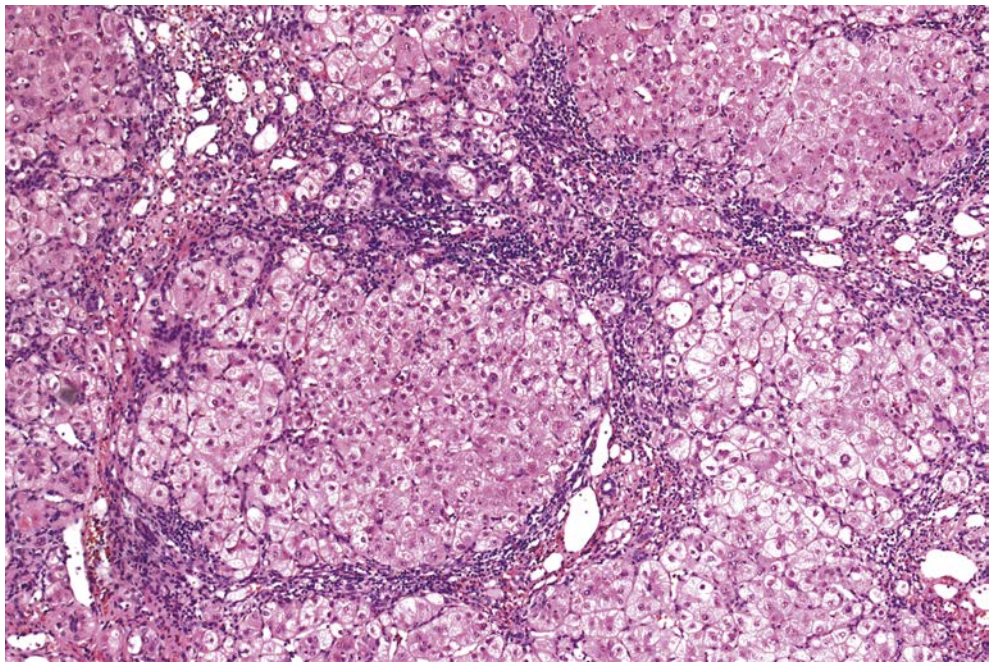

Fig. 10.20 Active cirrhosis. The outline of the nodule is blurred by interface hepatitis and there is a heavy inflammatory infiltrate. (Wedge biopsy, H&E.)

Complications

Hypoperfusion leads to coagulative necrosis involving whole nodules or their centres.[31] This is sometimes referred to as **nodular infarction**.

In recent years, advances in imaging and examination of explanted cirrhotic livers after transplantation have led to extensive discussion of the pathology and nomenclature of nodules different in appearance from the rest, and usually larger in diameter. The relationship of these nodules to HCC has been debated, and is further discussed in **Chapter 11**. An international working party[49] has recommended that the old term *adenomatous hyperplasia* should no longer be used, and that the nodules should be subdivided into **large regenerative nodules (macroregenerative nodules)** and **dysplastic nodules**. The latter are further subclassified as low grade and high grade. The dysplastic nodules differ from macroregenerative nodules in their content of dysplastic (atypical) hepatocytes and their more expansile growth pattern. However, the distinction between macroregenerative nodules and low-grade dysplastic nodules is often difficult, as is the distinction between high-grade dysplastic nodules and well-differentiated **HCC**, a very important complication of cirrhosis. In addition to the three large nodule types described by the working party, the group also defined **dysplastic foci**, clusters of dysplastic hepatocytes less than 1 mm in diameter.[50] In making a microscopic diagnosis and differentiating between these various possibly preneoplastic nodules, the pathologist must bear in mind that needle biopsy samples of a nodule may not be representative of the entire nodule, and that HCC may have arisen in a part not sampled by the needle.

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CHAPTER 11

Neoplasms and Nodules

Introduction

This chapter is intended to provide a working overview of the tumours and tumour-like nodular lesions that the pathologist will encounter with some frequency in everyday practice. The majority of these can be classified according to the putative cells of origin (hepatocytes, bile-duct epithelium and endothelium) from which they arise (**Table 11.1**), and immunohistochemistry can often be used effectively to distinguish histogenesis.[1] It is also germane to note that many liver tumours, both benign and malignant, now have known associations with genomic alterations that can be correlated with histologic features and are potentially useful in therapy[2–8] (**Table 11.2**). Neoplastic and nodular lesions of adults are covered first, followed by lesions in children and a section on cytopathological diagnosis. The reader is encouraged to consult the references and general reading list for additional details and coverage of some of the rarer tumours.

Table 11.1 Classification of liver tumours and nodular lesions.

Putative cell of origin	Benign	Malignant
Hepatocyte	Liver-cell adenoma MRN FNH NRH PNT	Hepatocellular carcinoma Fibrolamellar carcinoma Hepatoblastoma
Bile-duct epithelium	Bile-duct adenoma Cystadenoma Adenofibroma	Cholangiocarcinoma Cystadenocarcinoma
Mixed liver cell and bile-duct cell	Mesenchymal hamartoma	Combined hepatocellular cholangiocarcinoma
Endothelial cell	Haemangioma Infantile haemangioendothelioma*	Angiosarcoma Epithelioid haemangioendothelioma

FNH, focal nodular hyperplasia; MRN, macroregenerative nodule; NRH, nodular regenerative hyperplasia; PNT, partial nodular transformation.
*Some cases may behave more aggressively and are capable of metastasis.

Table 11.2 Genomic changes associated with liver tumours.

Tumour	Genomic change (% of cases)
Hepatocellular carcinoma[2]	*TERT* promoter mutations (60%) *TP53* mutation (30%) WNT signalling (*CTNNB1* 30%; *AXIN1* 10%) Chromatin remodelling (*ARID1A* 10%; *ARID2* 5%)
Fibrolamellar subtype	*DNAJB1-PRKACA* fusion
Cholangiocarcinoma	*CDKN2A* mutation (47%) KRAS mutation (22% intrahepatic; 42% perihilar and distal) IDH1/IDH2 mutation (25% intrahepatic) FGFR2 aberrations/rearrangements (10%–16% intrahepatic) HER2 amplification (11%–20% peripheral and distal; 4.8% intrahepatic[7])
Hepatocellular adenoma[4]	*HNF1A* inactivating mutation (*Steatotic adenoma*) β-Catenin activating mutation (*Atypical adenoma*) *JAK/STAT* pathway activation by somatic mutations (*inflammatory adenoma)*: -----*IL6ST* (encodes gp 130; 60% of mutations) -----*STAT3* (5% of mutations) -----*FRK* (10% of mutations) -----*JAK1* (3% of mutations) -----*GNAS* (5% of mutations) No known genomic change (*Unclassified adenoma*; 10% of cases)
Hepatoblastoma[5]	WNT signalling (*CTNNB1/APC*) with further subgroups: -----HNF1α; *Notch/PTEN* mutations (*favourable prognosis*) -----EpCAM/Lin28B/Let7/SALLA/HMGA2/AFP high/NRF2 (mutant or activated); MLL2/ARID1a (*unfavourable prognosis*) -----TP53 and RAD17 deletions/TERT promoter mutations/P13K/AKT (*possible transitional liver-cell tumour or hepatocellular carcinoma*)
Mesenchymal hamartoma[6]	C19g13.4 translocations

ARID1, AT-rich interactive domain 1; C19MC, chromosome 19 microRNA cluster; *CDKN2A*, cyclin D kinase 2A; *CTNNB1*, catenin beta-1; *FGFR2*, fibroblast growth factor receptor 2; *GNAS*, guanine nucleotide binding protein α-stimulating; *IL6ST*, interleukin 6 signal transducing; *JAK*, Janus kinase; *STAT*, signal transducer and activator of transcription.

Neoplasms and nodules in adults

Benign lesions

Hepatocellular adenoma

Hepatocellular adenomas (HCAs) are solitary or occasionally multiple tumours composed of hepatocytes. Macroscopically they are well defined but often not encapsulated. The cells of the tumour closely resemble normal hepatocytes (**Fig. 11.1**). Nuclei are small and regular and mitoses are almost never seen. These features are evident in fine-needle aspiration biopsies (FNABs).[9] The cells are arranged in normal or thickened trabeculae interspersed with prominent arteries and thin-walled blood vessels. In adenomas, reticulin is normal or sometimes reduced, but extensive loss is in most cases confined to areas of necrosis or haemorrhage. The latter are characteristically found in adenomas in oral contraceptive

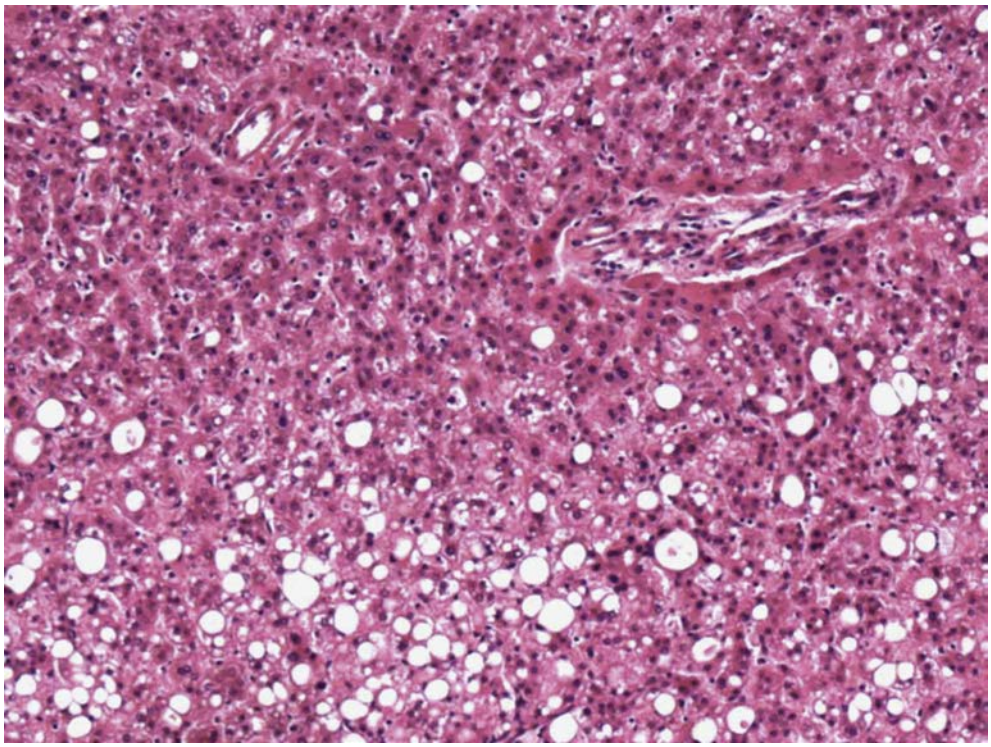

Fig. 11.1 Hepatocellular adenoma, steatotic type. Liver cells appear normal or contain fat vacuoles. Isolated blood vessels (upper left) or vessels within small amounts of connective tissue, but without accompanying bile ducts (pseudo-portal tracts; upper right) are seen within the lesion. (Operative specimen, H&E.)

users, and are responsible for pain and for the serious complication of haemoperitoneum. They probably also explain the fibrous scars which are sometimes found in the lesions. Regular septa, portal tracts and bile ducts are, however, absent; this distinguishes HCAs from both non-neoplastic liver and macroregenerative nodules (MRNs: large regenerative nodules) in cirrhosis and from focal nodular hyperplasia (FNH). Exceptions to this rule may occur in patients with multiple adenomas (adenomatosis[10]) where bile ducts can become entrapped within the lesions[11] and in the inflammatory adenoma where focal ductular reaction is sometimes present (discussed later).

Adenomas may contain Dubin–Johnson-like pigment[12] or show steatohepatitis with Mallory–Denk bodies.[13] Non-necrotising granulomas within adenomas are also described.[14,15]

Genetic–histological correlations have allowed subclassification of adenomas into several subtypes with distinctive immunohistochemical signatures (**Table 11.3**).[16–22] Approximately 30%–40% of adenomas show *HNF-1α* inactivating mutations, and these typically contain fat but show no cytological atypia[18–23] (**Fig. 11.1**). Activating β-catenin gene mutations are seen in some 10%–15% of adenomas with cytological atypia and acini,[24] and these tumours are more likely to show transformation to hepatocellular carcinoma (HCC) and the chromosome gains and losses seen in HCC.[25] Transformation is more common in men, and metabolic syndrome appears to be a risk factor.[26] The third, and most common, subtype of adenoma is the inflammatory adenoma (**Fig. 11.2**), which shows small amounts of connective tissue (pseudo-portal tracts) containing chronic inflammatory cell infiltrates (occasionally with adjacent ductular reaction) and/or sinusoidal dilatation. This subtype has been linked to *IL6ST, STAT3* and *GNAS*-activating gene mutations[24] and increased interleukin-6 signalling[27] and represents some 40%–50% of adenomas. Individuals with inflammatory adenomas may have a systemic inflammatory, flu-like syndrome or, very rarely, develop systemic AA amyloidosis.[28] The last HCA subtype accounts for 10% of adenomas, shows no unique histological or immunohistochemical

Table 11.3 Diagnostic distinctions between hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH).

Lesion	Routine diagnostic features	Key immunohistochemical stain(s)
HCA	Benign-appearing hepatocytes Thickened cords and trabeculae Interspersed venules and arterioles Absence of bile ducts	
HCA Subtype (%)		
(Gene mutation)		
Steatotic (30%–40%) (*HNF-1A* inactivating mutation)	Macrovesicular steatosis No cytological atypia	**LFABP:** absent (compared to positive in normal liver)
β-catenin (10–15%) (*β-catenin* activating mutation)	Nuclear atypia Acini	**β-Catenin:** nuclear and/or cytoplasmic positivity **GS:** diffuse, strong positivity
Inflammatory (40%–50%) *[activating mutations in IL6ST (codes for gp130), STAT3 and GNAS]*	Inflammation in pseudo-portal tracts Sinusoidal dilatation/ectasia may be present Focal ductular reaction may be present Steatosis sometimes present	**SAA:** cytoplasmic positivity **CRP:** cytoplasmic positivity
Unclassified (10%)	No distinctive features	None identified
FNH	Central stellate scar Thick-walled artery within scar Ductular reaction at edge of scar Cirrhosis-like nodular parenchyma	**GS:** Map-like broad fields of cytoplasmic positivity

CRP, C-reactive protein; GNAS, guanine nucleotide binding protein (G protein), alpha stimulating activity polypeptide; IL6ST, interleukin 6 signal transducer; LFABP, liver-type fatty acid protein; SAA, serum amyloid A; STAT3, signal transducer and activator of transcription 3.

features and is as yet genomically unclassified. The percentage of each adenoma subtype may vary depending on the population studied.[29] Genomic data have also stratified adenomas according to specific features such as risk factors, bleeding and tendency towards malignant transformation.[30]

Distinguishing adenoma from either FNH or well-differentiated HCC can be diagnostically challenging. Targeted use of immunohistochemical stains may be necessary for such distinctions (**Table 11.3**).[31] In the case of adenoma versus HCC, loss of reticulin, nuclear atypia and mitotic activity and the presence of many acinar structures favour carcinoma. Immunohistochemical demonstration of nuclear and/or cytoplasmic β-catenin overexpression is often helpful evidence of transition to carcinoma, but is not invariably present.[32] The presence of lipofuscin pigment within an adenoma (*pigmented hepatocellular* adenoma; **Fig. 11.3**) also warrants close pathological attention, because this subgroup, especially in males, is at increased risk of atypia and malignancy. Pigmented adenomas may be found in all histological phenotypes of adenoma, with the *HNF-1α* subtype the most common.[33] HCAs present diagnostic dilemmas, not only because of overlapping features in common

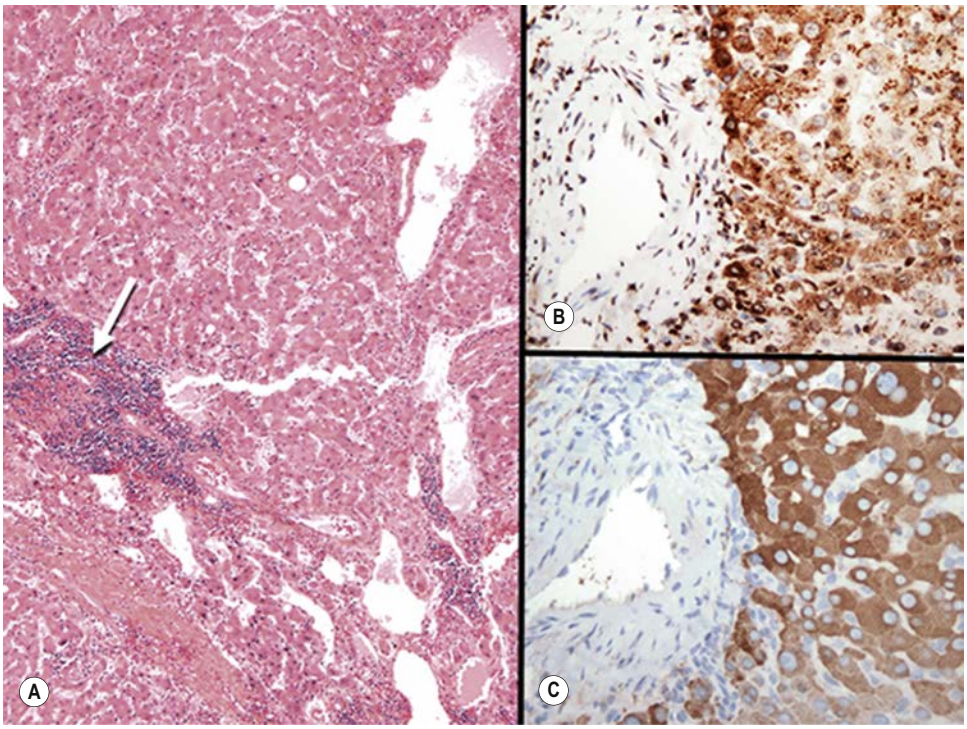

Fig. 11.2 Hepatocellular adenoma, inflammatory type. A: The histological hallmark of this type of hepatocellular adenoma is the presence of scattered lymphocytic inflammation within pseudo-portal tracts (arrow), accompanied by dilated, telangiectatic blood vessels and sinusoidal spaces. **B:** This tumour typically shows immunohistochemical positivity for markers of acute phase serum markers of inflammation, including C-reactive protein (B) and serum amyloid A (C). (Operative specimen: **A**: H&E; **B** and **C**: specific immunoperoxidase.)

with other benign hepatic lesions, but also because of overlapping features within subtypes of adenomas. Steatosis is not limited to the type 1, *HNF1-α* mutated adenoma, but may also be present in inflammatory adenomas. Ductular reaction is seen in FNH, but also, to a limited degree, in some inflammatory adenomas. Careful inspection of immunostains usually provides the critical distinction(s) between adenoma subtypes.[34] In difficult cases where concern exists regarding transition to or documentation of HCC, immunostain results may be insufficient (as in some cases with β-catenin activation where there is variant glutamine synthetase (GS) expression), and genomic evaluation, particularly of exon 3 of the *CTNNB1* gene, may clarify the diagnosis.[35] Atypical histologic features such as focal reticulin loss, pseudoglands (acini) and other variant data may require a diagnosis of *atypical hepatocellular neoplasm*, or, for example in lesions that develop in androgen users, the diagnosis of *HUMP (hepatocellular neoplasm of uncertain malignant potential*).[36]

Most HCAs arise in women of child-bearing age, usually after prolonged use of oral contraceptives.[37] Use of anabolic/androgenic steroids is a risk factor for both adenoma and HCC,[38] particularly in Fanconi's anaemia.[39,40] Rarely, adenomas arise in chronic liver disease and cirrhosis, usually the inflammatory subtype.[41] **Adenomatosis**,[42,43] in which multiple tumours are seen throughout the liver, is much less common, is associated with *HNF-1α* mutations and shows female predominance.[10] A subgroup of these cases is familial and associated with diabetes.[10,43–45] Adenomatosis is also seen in patients taking anabolic/androgenic steroids[46] or in patients without risk factors.[47] HCAs may also arise in patients with diabetes[48] or type I glycogen storage disease[49] (usually the inflammatory subtype[50])

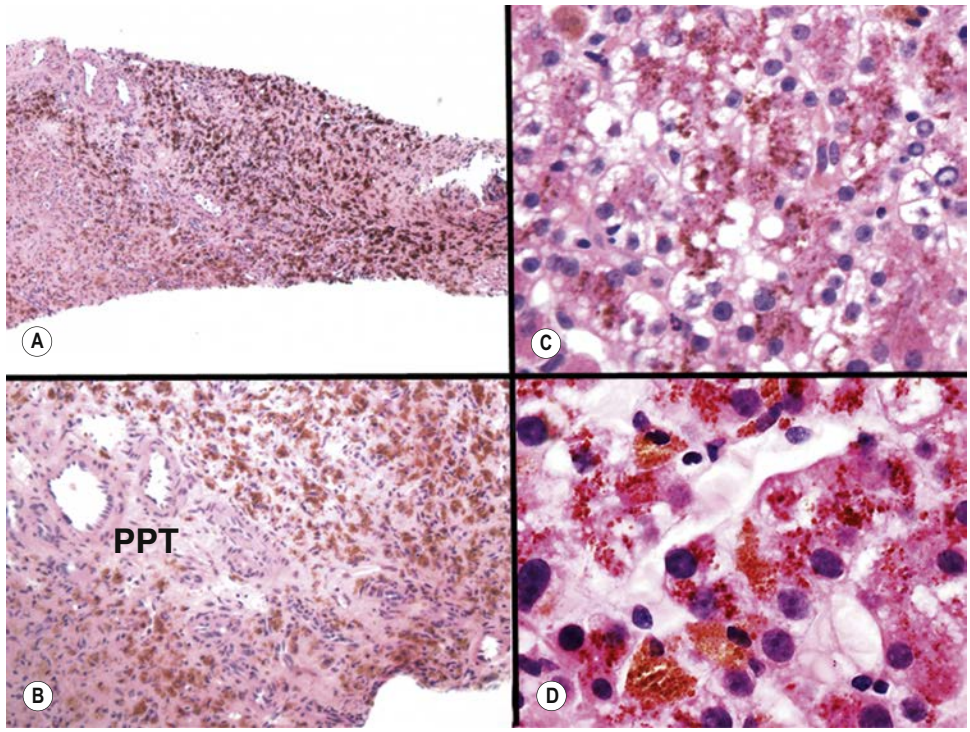

Fig. 11.3 Pigmented hepatocellular adenoma. A: Hepatocellular adenomas with lipofuscin pigment can be exceptionally striking on low magnification, sometimes suggesting the presence of malignant melanoma. **B:** Pigmented adenomas show otherwise general features of hepatocellular adenomas, including pseudo-portal tracts (PPT) with isolated arterioles and no bile ducts. **C:** Pigmented adenomas may be found among the major histologic phenotypes of hepatocellular adenomas, including steatotic, atypical and inflammatory subtypes. Note the focal steatosis in this example. **D:** Pigmented hepatocellular adenomas contain lipofuscin, as identified on transmission electron microscopy. (Operative specimen, H&E.)

and in children or young adults (see the 'Neoplasms and nodules in children' section). In older and elderly men, metabolic syndrome is of growing concern for the development of adenomas and possible evolution to HCC.[51]

Focal nodular hyperplasia

FNH is a fairly common lesion, seen in either sex and at any age. FNH is a reactive, hyperplastic response of polyclonal[52] hepatocytes, fibrous stroma and bile ductules due to a putative pre-existing arterial malformation.[53–56] FNH, unlike liver-cell adenoma, does not appear to be caused by oral contraceptives. Although oral contraceptives may cause an increase in size and vascularity,[57] they do not appear to influence the number or size of these lesions.[58] Bleeding and rupture are rare, as is recurrence after resection.[59] Features of FNH and adenoma are only very occasionally seen in the same tumour, and the occurrence of the two lesions in the same liver may be coincidental.[60] There may be multiple FNHs in the same patient, and such individuals often have other lesions, including vascular anomalies (hepatic haemangioma, telangiectasis of the brain, berry aneurysm, dysplastic systemic arteries, portal-vein atresia), central nervous system neoplasms (meningioma, astrocytoma)[61,62] and hemihypertrophy.[63]

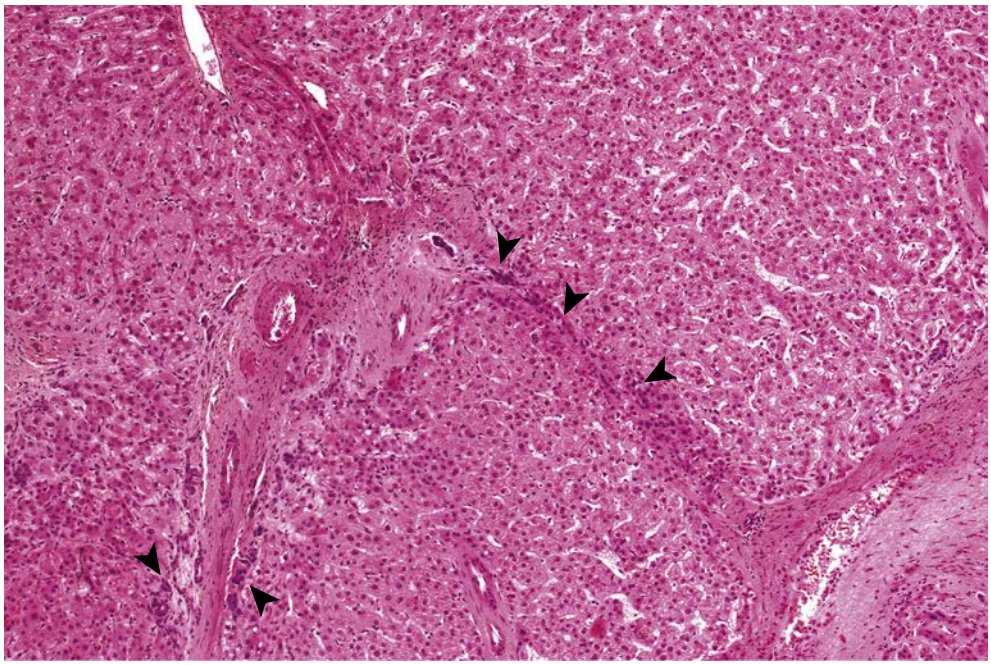

Fig. 11.4 Focal nodular hyperplasia. Part of a central scar with abnormal arterioles has been sampled. Radiating fibrous septa show small bile-duct-like structures at their edges (arrowheads). The parenchyma is nodular. (Operative specimen, H&E.)

Macroscopically, the nodules are well demarcated from the normal hepatic parenchyma. They are usually pale, and are dissected by fibrous septa into nodules, giving them an appearance very like that of cirrhosis. There may be a prominent central fibrous scar (**Fig. 11.4**) with closely associated smooth-muscle actin immunostain-positive activated stellate cells.[64] Histologically, the appearance is also very like that of inactive cirrhosis. The dense fibrous septa contain large thick-walled and sometimes narrowed arteries, as well as bile-duct-like structures probably derived from metaplastic liver-cell plates[65] or from progenitor cells.[55] Cytokeratin 7 immunostain highlights the bile ductular structures (**Fig. 11.5**) and helps distinguish FNH from adenoma.[66] The presence of bile-duct cells in fine-needle aspiration cytology of FNH is helpful in distinguishing this lesion from HCC.[67] In radiologically guided needle biopsies, the pathologist should be made aware that a mass lesion is being sampled, because the proliferated bile-duct-like structures and reactive stroma may otherwise suggest the diagnosis of mechanical bile-duct obstruction[68] (**Fig. 11.5**).

Lesions that grossly resemble FNH are also occasionally seen in Budd–Chiari syndrome.[69] Microscopically, these masses show hyperplastic, regenerative nodules in combination with other features, including central scars and multiple arterial structures. Some vary histologically so as to suggest crossover lesions between large regenerative nodules, FNH and liver-cell adenoma.[70] They appear to result from hyperarterialisation of regions of decreased hepatic venous blood flow.[70,71] FNH is also seen after liver transplantation in allografts with vascular perfusion abnormalities.[72]

FNH and adenoma are sometimes difficult to distinguish because of certain shared histological features, including the presence of isolated arterioles, thickened and nodular hepatocellular parenchyma, fibrosis and (in the inflammatory adenoma) inflammation and ductular reaction. The map-like staining pattern of broad islands of parenchyma in FNH seen with GS immunostain is helpful in confirming FNH (**Fig. 11.5**).[73]

Nodular regenerative hyperplasia

In nodular regenerative hyperplasia (NRH) multiple hyperplastic parenchymal nodules with thickened liver-cell plates are seen but fibrosis is absent or slight[74] (**Fig. 11.6**). This

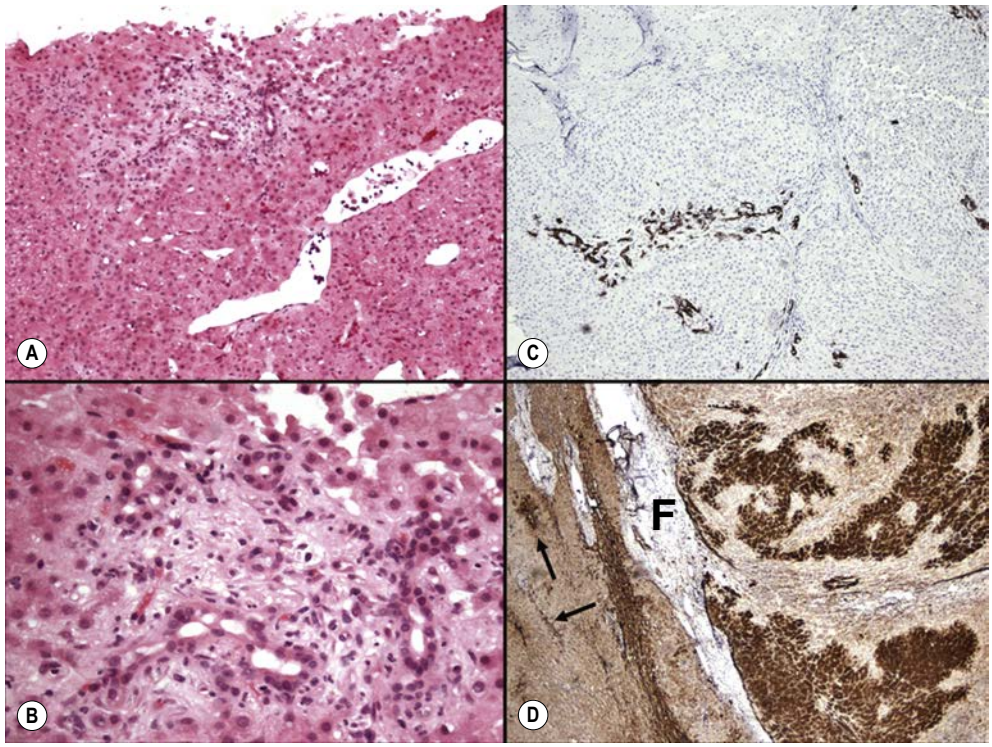

Fig. 11.5 Focal nodular hyperplasia (FNH). Needle biopsies of FNH are sometimes diagnostic problems due to the region sampled. **A, B:** A needle biopsy of FNH taken from near the fibrous scar and its associated ductular reaction could be mistaken for biliary obstruction. These fields highlight the oedematous stroma and ductular structures. **C:** Immunostain for cytokeratin 7 helps confirm the presence of ductular reaction as an important component of FNH. **D:** Immunostain for glutamine synthetase demonstrates the characteristic 'geographic' or 'map-like' pattern in the lesional parenchyma. Compare to the normal limited staining of centrilobular hepatocytes outside the lesion (arrows). F, central fibrous scar. (**A** and **B**: Needle biopsy, H&E; **C**: Operative specimen, specific immunohistochemistry; **D**: Operative specimen, specific immunohistochemistry.)

distinguishes the lesion from cirrhosis. In some cases perisinusoidal fibrosis is found in the compressed liver tissue between nodules. Portal tracts may be found at the centres of the nodules, but this is not invariable. Diagnosis is often difficult in needle-biopsy specimens. The nodularity may be more clearly seen in reticulin preparations (Fig. 11.7). A wedge liver biopsy may be required to establish the diagnosis and to exclude an important differential: incomplete septal cirrhosis (Ch. 10).

NRH is associated with a wide range of conditions, mainly rheumatic diseases, myeloproliferative disorders and chronic venous congestion.[75–77] While uncommon, NRH is not rare and may represent from 4% to 15% of liver biopsy specimens obtained per annum in the evaluation of abnormal serum liver function tests.[78] Patients with NRH may have received therapeutic drugs, including corticosteroids, anabolic steroids, oral contraceptives, antineoplastics,[79] anticonvulsants and immunosuppressive agents.[75,80,81] NRH has also been associated with HIV infection,[82] the toxic-oil syndrome,[83] Behçet's disease,[84] early histological stages of primary biliary cholangitis,[85] coeliac disease with anticardiolipin antibodies,[86] livers containing metastatic neuroendocrine tumours[87] and non-cirrhotic livers in which HCC has developed.[88] Some patients with NRH have portal hypertension, including (rarely) individuals with systemic mastocytosis.[89] Serum alkaline phosphatase and γ-glutamyl transpeptidase levels may be elevated.[77,85]

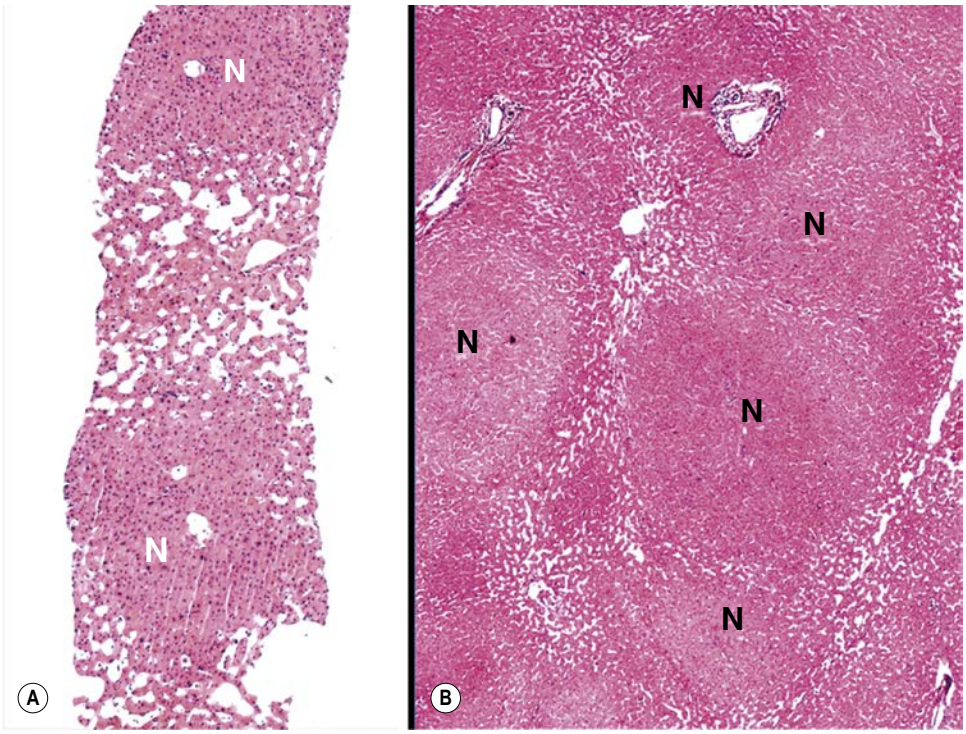

Fig. 11.6 Nodular regenerative hyperplasia. A: Needle biopsy showing obvious nodular regions (N) with intervening dilated sinusoids and focally compressed liver-cell plates. This abnormal, nodular growth pattern is not accompanied by fibrosis and therefore differs from cirrhosis. **B:** Wedge liver biopsy shows parenchymal nodules (N) which are often adjacent to or surrounding portal tracts. The intervening liver shows flattened and compressed liver-cell plates and sinusoidal dilatation. (**A** and **B**: H&E.)

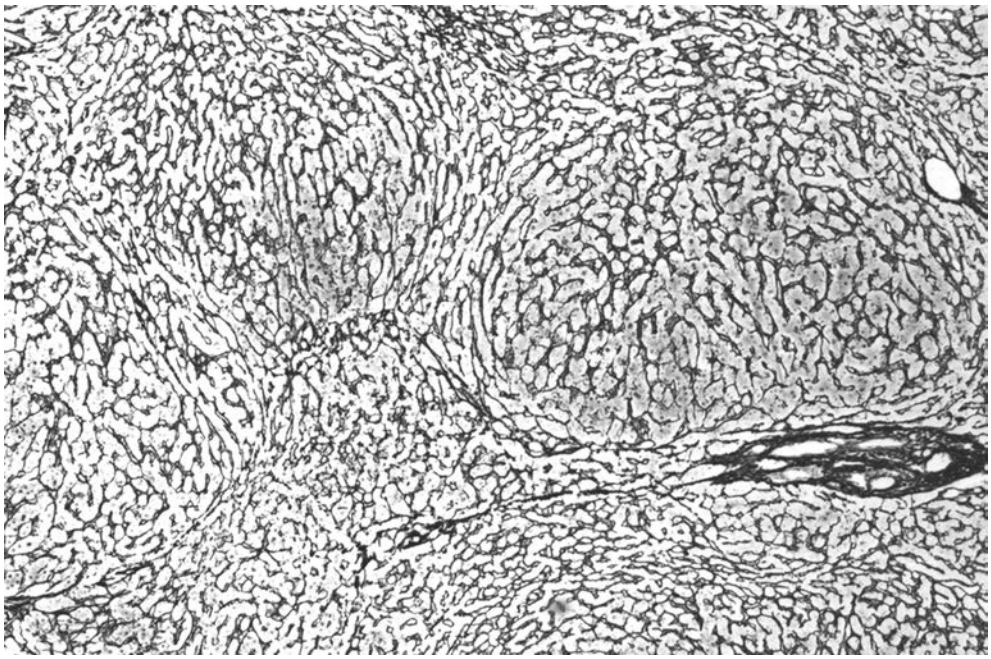

Fig. 11.7 Nodular regenerative hyperplasia. Reticulin stain of a field which highlights the regenerative nodules and the absence of fibrosis. (Postmortem liver, reticulin.)

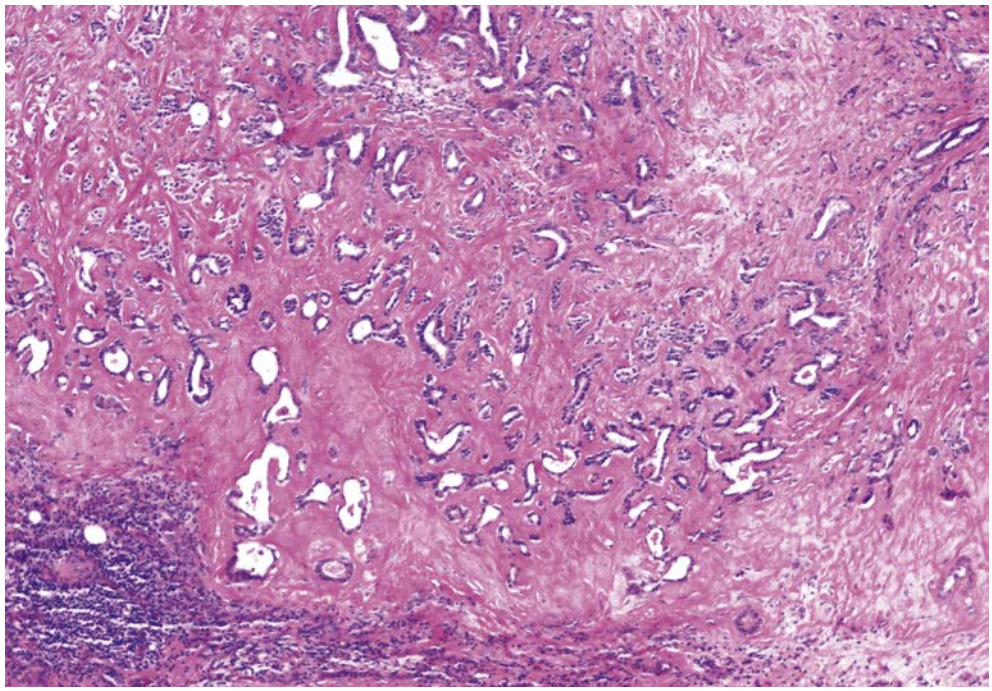

Fig. 11.8 Bile-duct adenoma. This subcapsular tumour consists of closely packed bile ducts set in a dense fibrous stroma. A dense collection of lymphocytes is seen at the edge of the lesion (bottom). (Operative specimen, H&E.)

Wanless and co-workers[90] have postulated that the basic lesion is portal venous thrombosis, leading to atrophy and compensatory hyperplasia. Arterial lesions, particularly arteriosclerosis of ageing, may also contribute to these changes.[77] Sinusoidal injury *per se* is another possible cause. Drug-induced sinusoidal injury, as seen with NRH development after oxaliplatin administration, is an example.[78,91,92] Disruption of fundamental cell biologic processes including telomere integrity and Notch1 signalling in the pathogenesis of NRH has received recent attention.[93] Short telomere syndrome has been associated with NRH, dyskeratosis congenita and common variable immunodeficiency[94] as well as with cryptogenic cirrhosis and idiopathic pulmonary fibrosis.[93] Disruption of Notch1 signalling events involving endothelium in adult mice as well as Notch1 haploinsufficiency and downregulation also result in the development of NRH. Portal venous thrombosis has also been invoked in the pathogenesis of the rare **partial nodular transformation**, in which somewhat larger nodules are found, often localised to the perihilar region, where they may cause portal hypertension.[95,96] NRH, FNH and partial nodular transformation share the common feature of liver-cell hyperplastic growth in the form of nodules; they have accordingly been grouped under the umbrella heading of 'nodular transformation' by Wanless.[97]

Bile-duct adenoma

Bile-duct adenomas are small, grey-white, usually subcapsular nodules measuring from 1 to 20 mm in diameter,[98] which may represent hamartomatous peribiliary glands or reactive biliary lesions with features of foregut pyloric metaplasia, rather than a neoplasm.[99,100] They are more often solitary than multiple. Histologically, they are composed of small, well-formed ducts embedded in a stroma of mature fibrous tissue which may contain chronic inflammatory cells, often densely aggregated at the periphery of the lesion[98,101,102] (**Fig. 11.8**). Their chief importance is that they may be mistaken for metastatic carcinoma, both macroscopically and microscopically. They differ from microhamartomas (von Meyenburg complexes) in that the ducts are smaller and more numerous, are usually not dilated and do not contain bile.[98,103] Periodic acid–Schiff (PAS)-positive, diastase-resistant globules of α_1-antitrypsin

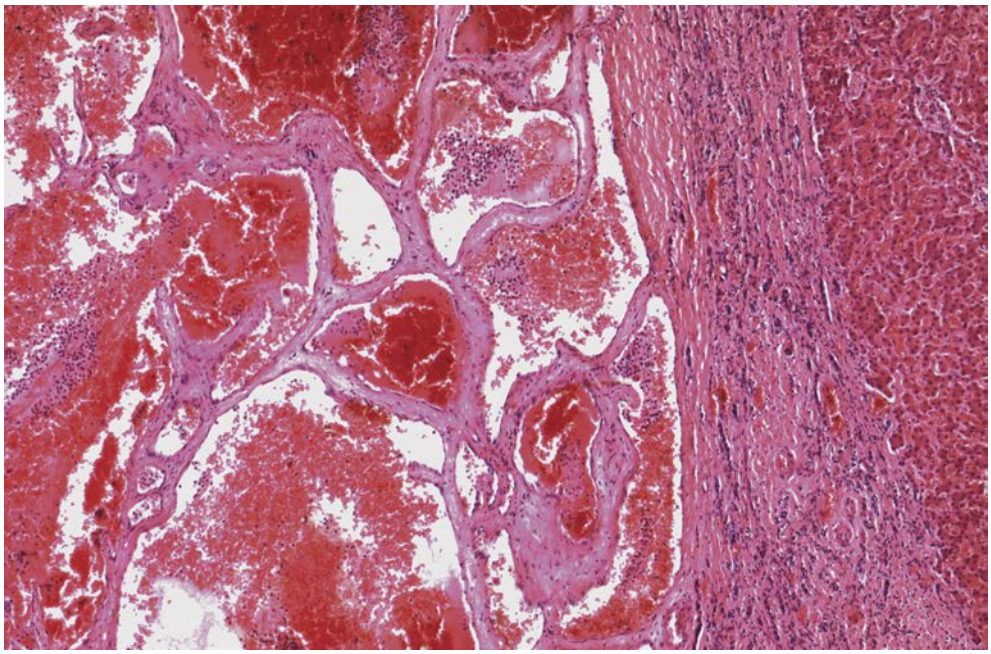

Fig. 11.9 Haemangioma. Blood-filled spaces are separated by fibrous septa. A thick capsule is seen at right. (Operative specimen, H&E.)

within the bile-duct epithelium of multiple adenomas were described in a patient with heterozygous α_1-antitrypsin deficiency.[104] The bile-duct adenoma should also be distinguished from the rare **biliary adenofibroma**, a much larger tumour composed of tubulocystic bile-duct structures with apocrine metaplasia and intraluminal bile embedded in fibrous stroma, resembling fibroadenoma of the breast.[105]

Biliary cystadenoma

Biliary cystadenoma is a multilocular tumour, the cystic spaces of which contain mucoid fluid and are lined by columnar, mucin-secreting epithelium which may form papillary projections. A variant **hepatobiliary mucinous cystic neoplasm** with subepithelial **ovarian-type stroma** occurs in women.[106–108] Malignant change is uncommon.[108]

Haemangioma

The cavernous haemangioma is the most common benign tumour of the liver, found incidentally at autopsy or operation and occasionally seen in biopsy material.[109] A few reach a large and clinically significant size. As in other sites, the lesions are composed of endothelium-lined channels supported by a fibrous stroma (**Fig. 11.9**). Lesional tissue sometimes extends irregularly into adjacent liver.[110] Complications include thrombosis, sclerosis and calcification.[111] Sclerosed haemangiomas may present diagnostic difficulties on needle biopsy and can be confused with healed granulomas, arteriovenous malformations or non-specific hepatic scars. Immunohistochemistry for endothelial cell markers (CD34 or CD31) usually allow a definitive diagnosis to be made (**Fig. 11.10**). Spontaneous rupture is recorded but uncommon. A distinction should be made between cavernous haemangiomas and peliosis (**Ch. 12**); the latter lacks the complete endothelial layer and fibrous trabeculae.

Rare, infiltrative vascular tumours in adults designated **hepatic small vessel neoplasms** have been described that appear to be low-grade, benign vascular tumours lined by endothelium with ovoid-to-plump nuclei and immunohistochemical positivity for CD34,

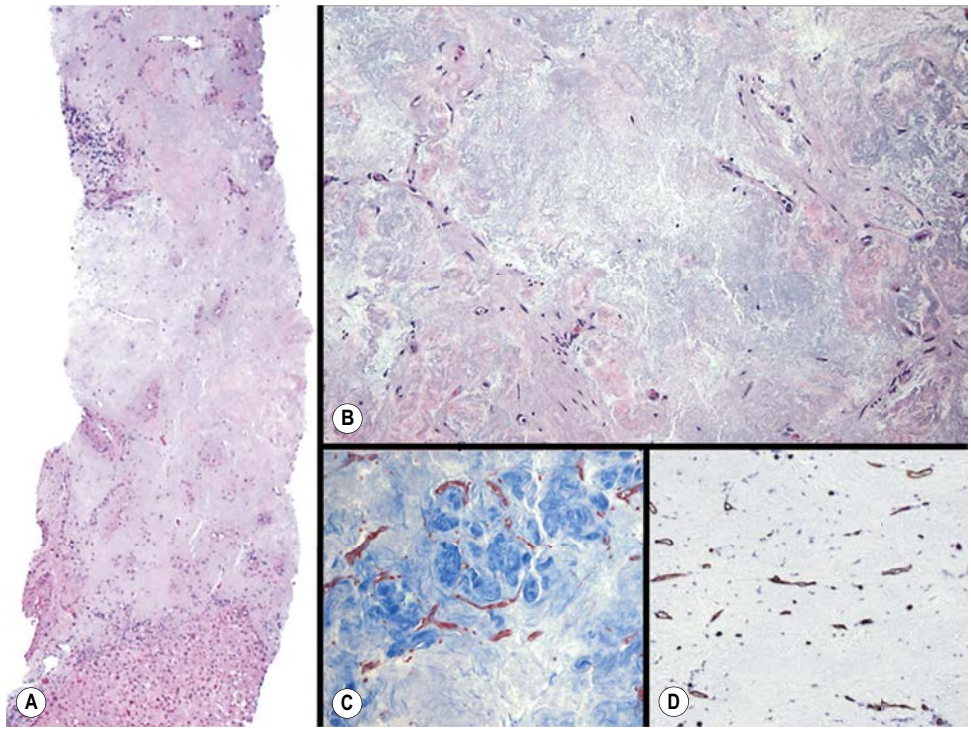

Fig. 11.10 Sclerosed haemangioma. A: Needle biopsy of a liver mass shows a densely fibrotic tumour without an otherwise specific pattern. **B:** Dense collagen with slit-like, flattened spaces is present. **C:** Oval-shaped, densely collagenized collagen bundles between slit-like spaces represent sclerosed vascular lumens. **D:** CD34 immunostain for endothelium confirms the presence of vascular spaces within the sclerotic stroma. (Needle biopsy: **A** and **B**: H&E; **C**: Masson trichrome stain; **D**: specific immunoperoxidase.)

CD31 and the proto-oncogene FLI-1.[112] However, they demonstrate cellular proliferative indices >10% with Ki-67 immunostain and histologically show borders with lesional vessels infiltrating adjacent sinusoids. **Lymphangioma** of the liver has been reported as part of multiorgan lymphangiomatosis or as a solitary hepatic lesion,[113] but is very rare. The endothelium-lined channels of this neoplasm are empty or contain lymph with occasional leukocytes. It should not be mistaken for mesenchymal hamartoma (see the 'Neoplasms and nodules in children' section).

Mesenchymal and neural tumours

Connective-tissue elements, adipocytes and smooth muscle of the liver, nerve sheaths of intrahepatic nerves and other mesenchymal cells may give rise to rare tumours, including lipomas, myelolipomas, angiomyelolipomas,[114,115] schwannomas and neurofibromas,[116–118] solitary fibrous tumours[119] and chondromas.[120] **Angiomyolipomas** resemble their more common renal counterparts and contain blood vessels, smooth muscle (myoid cells) and fat.[121] These components allow subcategorisation into mixed, lipomatous, myomatous and angiomatous types, in decreasing order of frequency.[122,123] Multiple tumours may be present.[124,125] Muscle cells may be partly of epithelioid type, with finely granular eosinophilic cytoplasm and pleomorphic nuclei[126–129] (**Fig. 11.11**). These may be mistaken for hepatocytes or malignant cells, particularly in cases where the component of fat is minimal. Megakaryocytes and other bone marrow elements are commonly present.

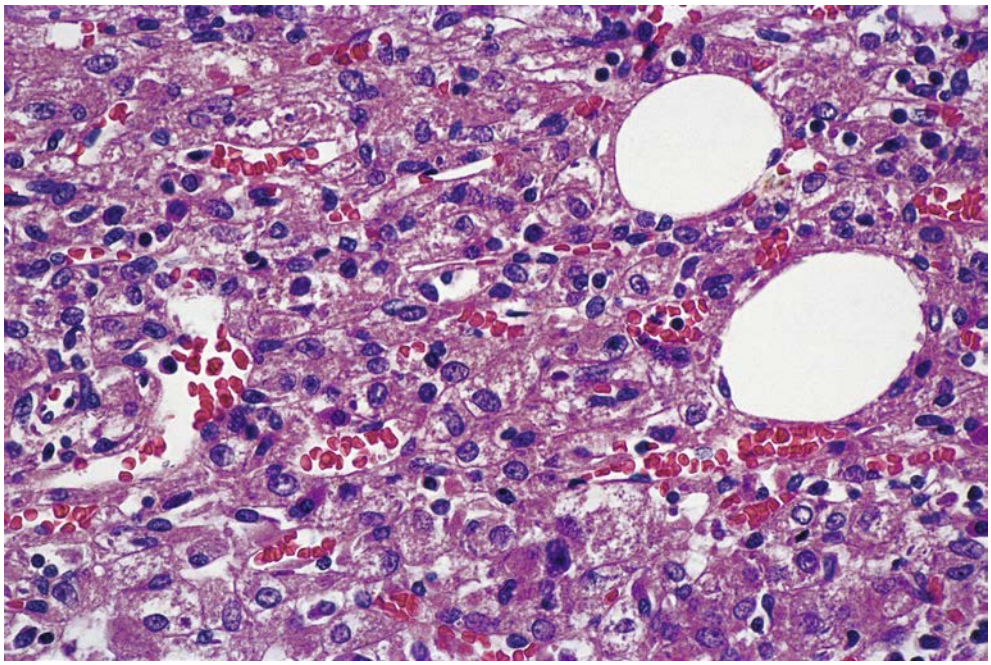

Fig. 11.11 Angiomyolipoma. The tumour shows myoid cells with ample granular cytoplasm resembling hepatocytes. Fat vacuoles (right) were variably scattered through the tumour, as were small blood vessels. (Operative specimen, H&E.)

Positive HMB-45 immunostaining of the myoid cells is a major diagnostic feature.[122,129] **Pseudolipomas**[130] probably represent separated nodules of peritoneal fat which become embedded in the liver capsule.

Inflammatory pseudotumour

Lesions of inflammatory pseudotumour may be solitary or multiple and usually occur in young, male patients with constitutional symptoms, fever and weight loss. They may sometimes involve structures near the porta hepatis with resultant biliary problems or portal hypertension, or may mimic HCC.[131] Surgical resection is the treatment of choice, when possible. The microscopic hallmark of inflammatory pseudotumour is the extensive polyclonal plasma-cell infiltrates which are intermixed with lymphocytes, eosinophils, foamy histiocytes and variable degrees of stromal proliferation, including spindle cells in bundles and whorls with associated fibrosis[132] (**Fig. 11.12**). Granulomas and partly obliterated blood vessels may be present. The lesion falls within a diagnostically controversial spectrum ranging from an inflammatory–reparative process (possibly infectious in aetiology) to a low-grade stromal malignancy termed *inflammatory myofibroblastic tumour*.[133,134] Some have been thought to be follicular dendritic cell tumours related to Epstein–Barr virus infection.[135] Immunostains are helpful to characterise individual cases. Among these, smooth-muscle actin will highlight the extent of the myofibroblastic component and activin-like kinase 1 expression in the spindle cells favours a diagnosis of inflammatory myofibroblastic tumour.[133,134] Infrequently, such lesions are part of the spectrum of IgG4-related disease[136–138] and show abundant IgG4-positive plasma cells with IgG4 immunostain.

Malignant lesions

Precursors of hepatocellular carcinoma

A number of hepatocellular changes and nodular lesions have been considered premalignant or precursors[139] of HCC, and these are discussed in the following section. Despite

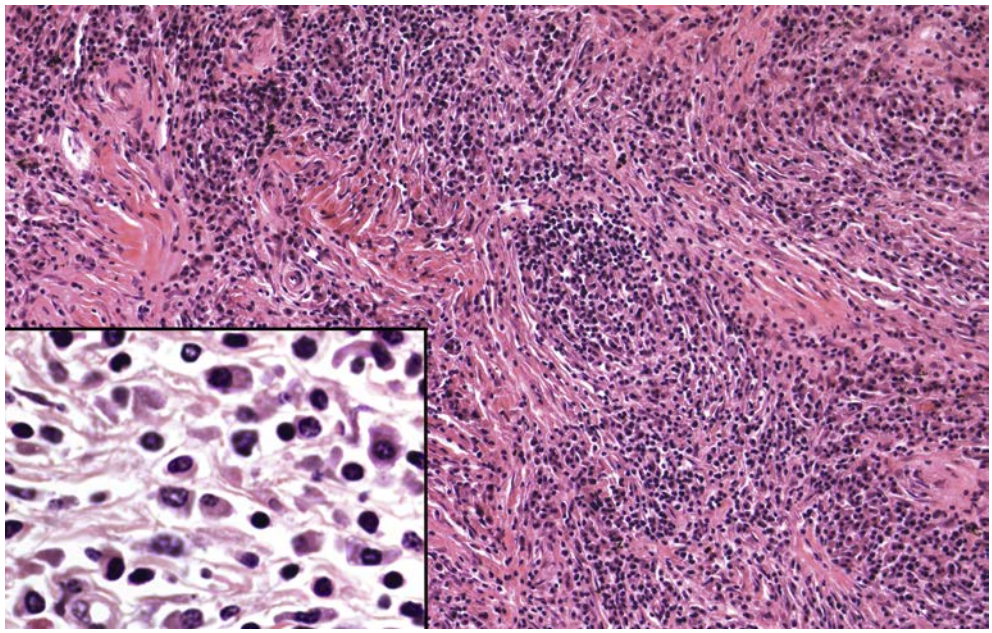

Fig. 11.12 Inflammatory pseudotumour. Dense infiltrates of plasma cells, lymphocytes and histiocytes with interwoven bundles of collagen are seen. Inset: Plasma cells are unusually prominent. (Operative specimen, H&E.)

refinements in the terminology of these lesions provided by panels of hepatic pathologists,[140–142] the precise sequence of histological and molecular changes in the presumed multistep pathogenesis of HCC in humans has not been established. The importance of recognition of these worrisome lesions is based on the need for close patient surveillance and possible surgical resection (or liver transplantation) once they are identified pathologically. The presence of one or more of these lesions should be clearly stated in the pathologist's report.

Non-neoplastic liver tissue may show varying degrees of **liver-cell dysplasia (LCD)** of either large- or small-cell type (**see Figs 10.8** and **10.9**). **Large-cell LCD (large-cell change)** is the type most often observed and features cell and nuclear enlargement, nuclear pleomorphism, multinucleation and multiple nucleoli and increased nuclear staining[143] (**see Figs 10.8 and 11.41**). Its distribution is random within lobules or cirrhotic nodules and should be distinguished from the variations in nuclear morphology seen in perivenular hepatocytes with ageing, in the presence of cholestasis or in methotrexate therapy. This type of dysplasia was first associated with hepatitis B virus infection, cirrhosis and HCC[144,145] and subsequently with a four- to fivefold increased risk of HCC in several studies.[146,147] Affected cells are usually aneuploid[148] and may have attendant chromosomal abnormalities.[149] However, it has been considered merely an effect of cholestasis[150] or a derangement in normal liver-cell polyploidisation[151] and has not been proven to be a direct pathogenetic precursor lesion of HCC. Nevertheless, it is a strong independent risk factor for the development of HCC[152,153] and thereby identifies patients requiring more diligent surveillance.

Small-cell LCD (small-cell change) is characterised by enlarged, hyperchromatic nuclei within small hepatocytes (increased nuclear–cytoplasmic ratio) arranged in crowded clusters[154] (**see Figs 10.9 and 11.42**). These foci show high cellular proliferation rates[155] and an overall cytological resemblance to HCC, and may originate from progenitor cells.[152] These features have lent support to small-cell LCD as a true precursor lesion that is subject to the later cellular events leading to the development of HCC.[156]

Other cellular changes cited as indicators of premalignancy include intracytoplasmic Mallory bodies,[157] irregular areas of regeneration showing hepatocyte glycogenosis, oncocytic change (**Ch. 9**) or bulging nodularity,[158] iron-negative foci in siderotic MRNs[159] and 'iron-free foci' in livers of patients with hereditary haemochromatosis[160];

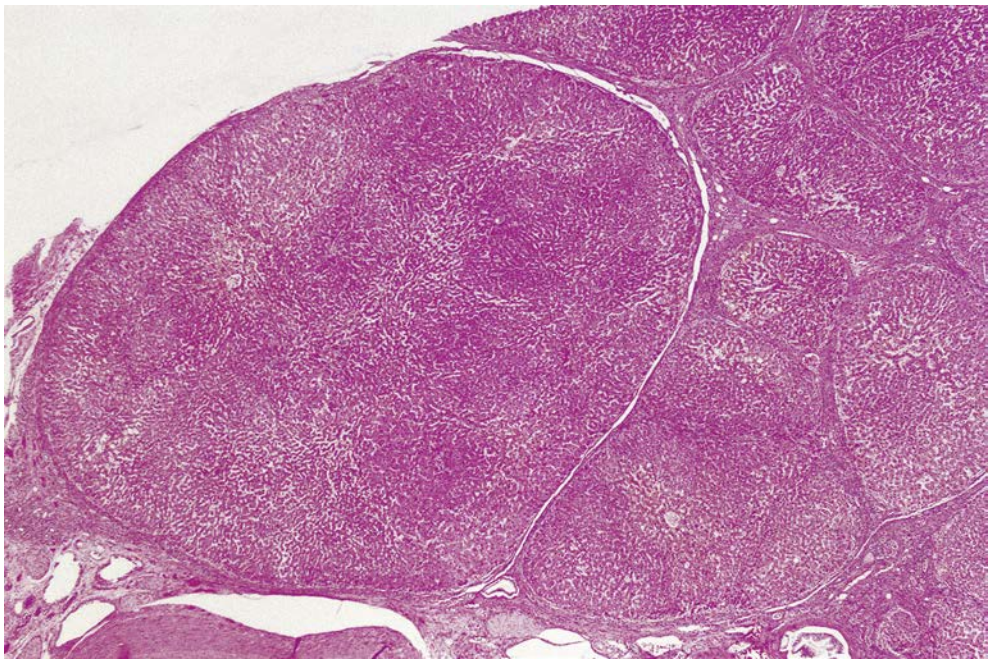

Fig. 11.13 Macroregenerative nodule. This low-magnification view demonstrates the increased size of the nodule at right compared with the cirrhotic nodules at left. (Operative specimen, H&E.) (Illustration kindly provided by Dr Kamal Ishak, Washington, DC, United States.)

the last may show large-cell LCD.[160] Clusters of large- or small-cell dysplastic hepatocytes less than 1 mm in diameter have been termed **dysplastic foci** by an international working party.[141]

The **MRN** is an unusually large regenerative nodule measuring 0.8 cm or more in diameter which develops in cirrhosis or other chronic liver disease[161] (**Fig. 11.13**). MRNs are particularly common in macronodular cirrhosis.[162] They may be paler or more bile-stained than the surrounding liver.[141] The cirrhotic liver may harbour several MRNs, which may coexist with HCC elsewhere in the liver or may contain foci of carcinoma. Cirrhotic explant livers should be carefully examined for these lesions[140,163] and for LCD.[164]

The MRN histologically shows hyperplastic liver parenchyma arranged in plates two or three cells thick, which is typical of cirrhosis. The nodule contains portal tracts and fibrous septa with bile ducts, hepatic arteries and portal-vein branches, and shows no cellular atypia or disorder in the liver-cell plate arrangement. Steatosis, haemosiderin, bile plugs and Mallory bodies may be present.[161,165] The terms 'adenomatous hyperplasia', a former synonym of MRN, and subdivisions into MRN types I and II[162] are not currently advocated for use.[141]

The **dysplastic nodule (borderline nodule)** shows atypical architectural and/or cytological features that are not acceptable for a benign MRN, but which fall diagnostically short of frank HCC.[141,166] Dysplastic nodules may show varying degrees of large- and small-cell LCD, increased cellularity and foci where the liver cords are less cohesive, focal loss of reticulin fibres or pseudoacini[140,141,163,166] (**Fig. 11.14**).

The major diagnostic concern is to distinguish MRNs and dysplastic nodules from HCC. Certain features seen in these nodules are associated with high risk of progression to carcinoma, including an increased ratio of nuclear density, clear-cell change, small-cell dysplasia and fatty change.[167] Increased mitotic activity, loss of reticulin fibres, formation of broad trabeculae and an infiltrative margin are helpful evidence of carcinoma.[140,163,168,169] Demonstration of clonality and loss of heterozygosity[170] and increased cell proliferative indices[171] are further supportive evidence of HCC.

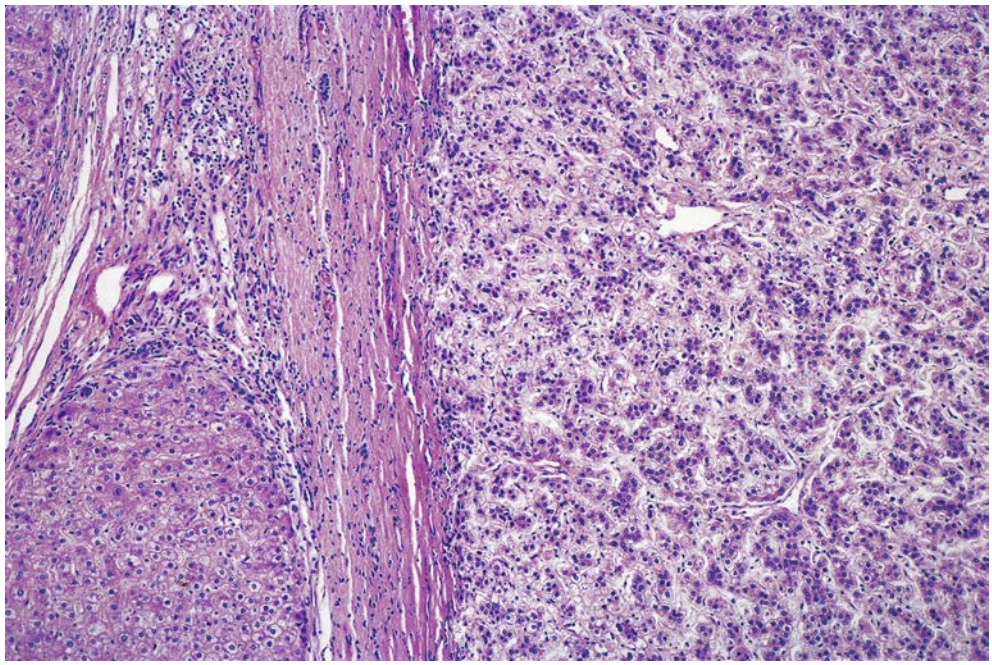

Fig. 11.14 Dysplastic nodule. The dysplastic nodule at right shows hepatocytes arranged in pseudoacini, with a less cohesive growth pattern centrally. A cirrhotic nodule is present at lower left. The patient had an inactive cirrhosis due to tyrosinaemia. (Explant liver, H&E.)

Hepatocellular carcinoma

HCC ranks sixth in incidence among malignant tumours worldwide and is the fourth most common cause of cancer-related death.[2] Epidemiological and other studies of HCC have defined geographical variations in the incidence and prevalence of this tumour, as well as a multifactorial aetiology.[172] Chronic necrosis and inflammation of the liver are important driving forces in the multistep process of hepatocarcinogenesis[173,174] in the context of underlying risk factors such as hepatitis B and C viral infections,[145] iron overload,[175] aflatoxin exposure[176] and the presence of fatty liver disease.[177–179] At the molecular level, identification of genetic changes that control cell cycling and apoptosis,[180] as well as oncogene expression,[181] gene deletions and amplifications,[182] mutation of tumour suppressor genes such as *p53*,[183] expression of vascular and cellular growth factors[184–189], somatic mutations in the *TERT* gene for telomerase reverse transcriptase proliferation of hepatic stem cells or their progeny[190–192] constitute a large and growing literature on this subject.

The majority of HCCs develop in cirrhotic liver.[193] The cause of the cirrhosis is usually known, even in many cases labelled as 'cryptogenic' where risk factors for non-alcoholic fatty liver disease (**Ch. 7**) become apparent.[178] The non-cirrhotic setting accounts for a substantial number of cases from North America[194] and elsewhere,[195] and can be seen in hepatitis B virus carriers[196,197] or in those with suspected occult hepatitis B,[195] in individuals infected with hepatitis C virus,[198] and, increasingly, in non-alcoholic fatty liver disease with large-droplet fatty liver.[199] In older and elderly non-cirrhotic men with metabolic syndrome and without cirrhosis, HCA may precede the development of HCC.[51] HCC may even develop within ectopic liver.[200] The cirrhosis associated with carcinoma is often macronodular in pattern, except for the micronodular cirrhosis seen in genetic haemochromatosis and chronic hepatitis C. The cirrhosis is usually inactive, although inflammation and necrosis may be seen near the tumour itself. Tumours may

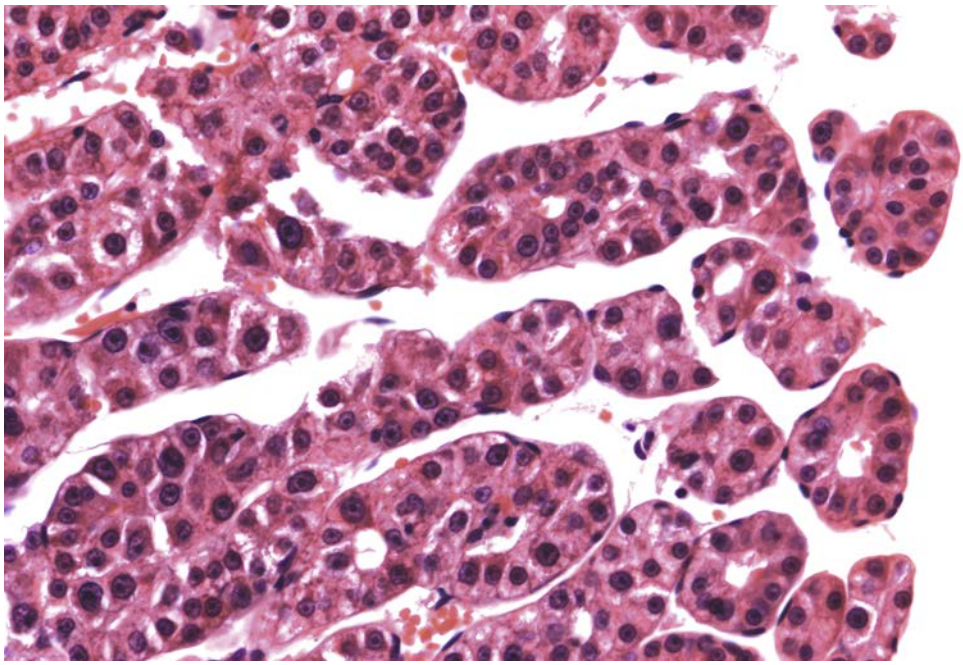

Fig. 11.15 Hepatocellular carcinoma. Note the trabecular–sinusoidal structure and resemblance of the tumour cells to normal hepatocytes. (Needle biopsy, H&E.)

be multifocal.[201] Intrahepatic tumour spread is both portal (via portal-vein branches) and lobular.[202] Rarely, HCC may spontaneously regress.[145,203,204] Following transplantation, cirrhotic explant livers require careful examination for small carcinomas and precursor lesions which are clinically undetected.[205] Pathology reports on explants or partial resections with HCC should specify the number of lesions and their sizes, as well as the histological grade and evidence of vascular invasion,[206] because these factors affect TNM staging and other prognostic classifications.[207]

The outstanding histological features of HCC are the resemblance of the tumour cells to normal hepatocytes, and of their arrangement to the trabeculae of normal liver (**Fig. 11.15**). However, the trabeculae are for the most part thicker, and reticulin is often scanty or even absent (**Fig. 11.16**). This paucireticulin pattern is even helpful in FNABs (see the 'Cytopathological diagnosis' section). In exceptional cases where there may be an increase in reticulin, other histological features and/or the clinical behaviour of the tumour must be used as diagnostic criteria of malignancy. Rarely, the trabecular pattern and even bile production are mimicked by primary tumours (hepatoid carcinomas) of the stomach, ovary and other sites[208–210] (see the 'Metastatic tumour' section). Between the tumour trabeculae in HCC there is a network of vascular channels lined by endothelium which is positive with immunostains for CD34,[184,185] factor VIII–related antigen and *Ulex europaeus* lectin.[211] The endothelial lining of these channels is a particularly helpful diagnostic feature in fine-needle aspirates. The absence of portal tracts and a cohesive connective tissue framework in the tumour results in a characteristic fragmentation of needle biopsy specimens with separation of tumour trabeculae that is readily observed at low magnification (**Ch. 4**, **Fig. 4.1**). Although connective tissue stroma is uncommon except in **fibrolamellar carcinoma** (described later), focal areas of fibrosis may follow tumour necrosis. In addition, a small percentage of HCCs are **scirrhous HCCs** (**Fig. 11.17**) and must be distinguished from fibrolamellar HCC, cholangiocarcinoma and metastatic carcinoma. The risk factors for this variant include chronic hepatitis B and C, steatosis and steatohepatitis (with or without cirrhosis).[212] The hepatocellular origin of the tumour may be obscured by its extensive constitutive fibrosis (therein resembling metastatic carcinoma) and its positivity for CK7

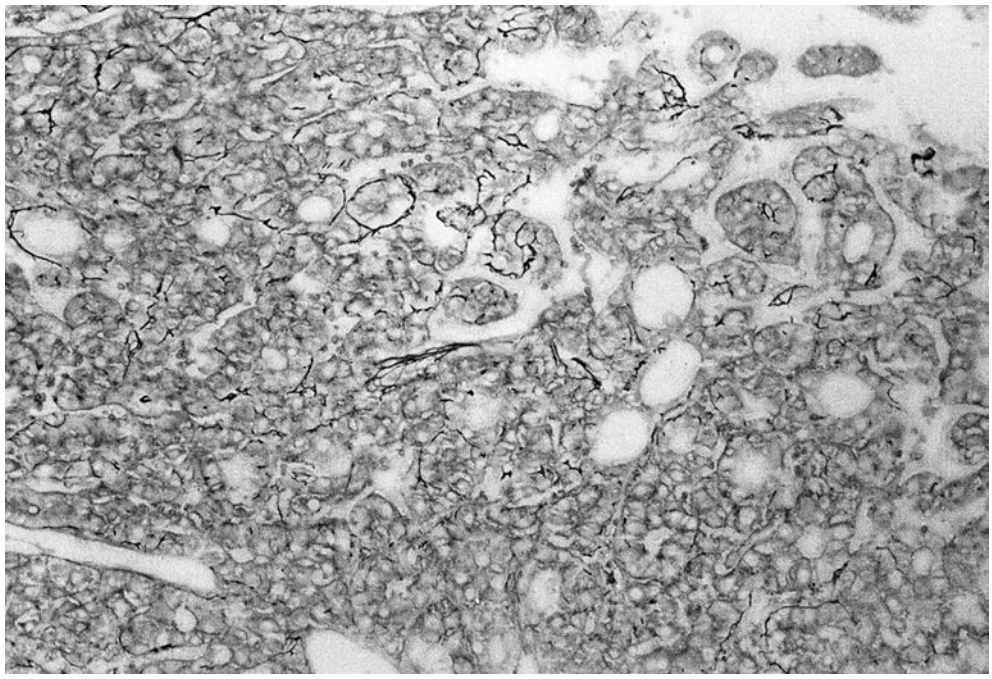

Fig. 11.16 Hepatocellular carcinoma. Reticulin is scanty in this example. (Needle biopsy, reticulin.)

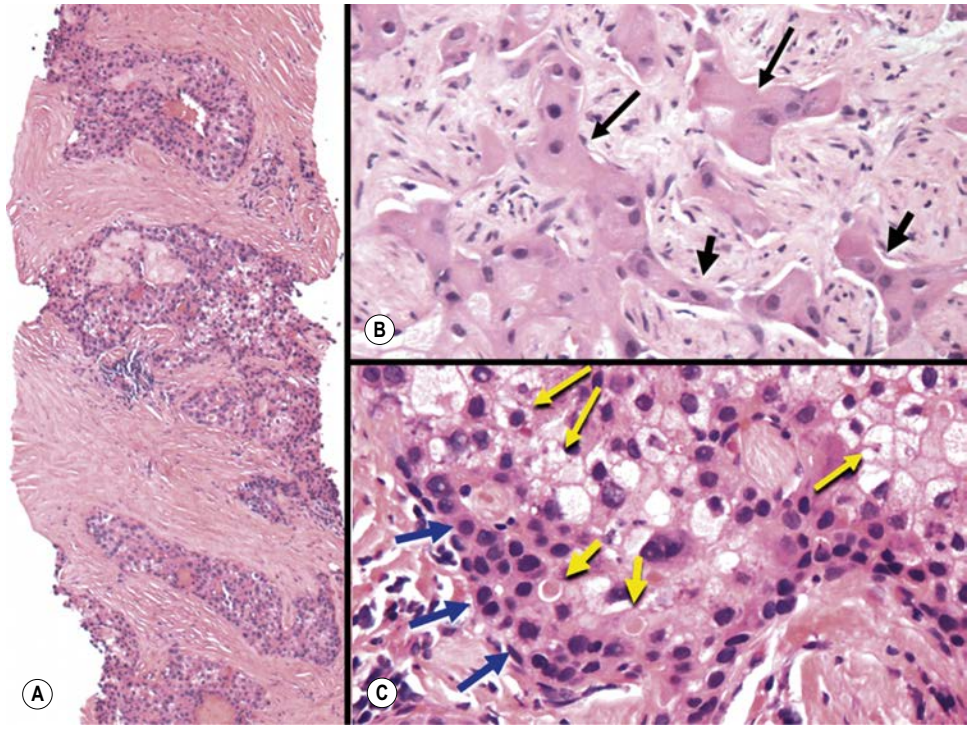

Fig. 11.17 Scirrhous hepatocellular carcinoma. A: The nests of tumour cells are surrounded by extensive fibrosis, resembling the desmoplastic response in many metastatic carcinomas. **B:** The neoplasm shows an admixture of cells with hepatocellular features (long arrows) and a flatter, more cuboidal cell population with biliary/stem cell features (short arrows). **C:** This nest of tumour cells shows focal intracellular hyaline bodies (short arrows), glycogenated, hepatocyte-like cells (long arrows) and peripheralized low cuboidal cells with higher nucleus-to-cytoplasmic ratio (blue arrows) with more resemblance to cholangiocytes or stem cell features (blue arrows). (Needle biopsy, H&E.)

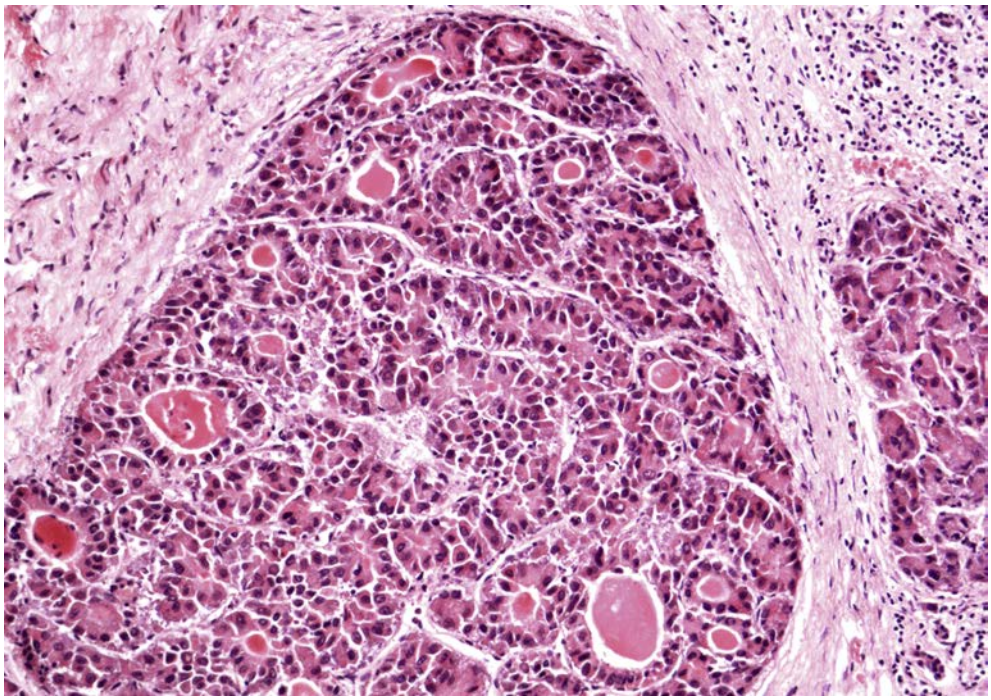

Fig. 11.18 Hepatocellular carcinoma. Adenoid pattern. Other areas of this tumour showed a more typical trabecular structure. (Postmortem liver, H&E.)

and negativity for Hep Par 1, but its identity can be confirmed with the combination of glypican-3 (GPC-3) and arginase-1 immunostains[212] (see further discussion of immunohistochemistry later). The neoplastic hepatocytes of scirrhous HCC may show steatosis, likely reflecting the association of this tumour with non-alcoholic fatty liver disease.[213] The nests and islands of tumour cells in scirrhous HCC may show biphasic features on haematoxylin and eosin (H&E), with peripheralisation of a low cuboidal cell population more resembling cholangiocytes than hepatocytes (and demonstrating biliary or stem cell immunohistochemical positivity) and more centrally based cells with hepatocellular features (**Fig. 11.17**).

The so-called **sclerosing carcinoma**[214] has been associated with hypercalcaemia but represents a poorly defined category in which some tumours may be of cholangiocyte origin. The **adenoid** (acinar) variant of HCC (**Fig. 11.18**) should not be confused with adenocarcinoma of the biliary tree. Bile-duct carcinomas are usually scirrhous, mucin-secreting tumours, whereas the characteristic secretion of HCCs is bile, seen in a minority of tumours in spaces homologous with normal bile canaliculi. The large repertoire of histological features of HCC also includes the 'steatohepatitic-HCC' (SH-HCC) variant which recapitulates many of the features seen in benign steatohepatitis[215] (**Fig. 11.19A**), the 'lymphoepithelioma-like' HCC (**Fig. 11.19B**) with admixed lymphocytes (predominantly T lymphocytes with fewer B cells) and variable association with Epstein–Barr virus[216–220] and the 'chromophobe HCC with abrupt anaplasia' variant.[221] Mixed or combined tumours designated **combined hepatocellular–cholangiocarcinoma** are also well described, with special stains and immunohistochemical features representative of both hepatocellular and bile-duct epithelial derivation.[222] Progenitor/stem-cell constituents are sometimes present[223] (discussed later).

At a cellular level variants include giant-cell forms with multinucleated tumour cells (a bad prognostic sign[224]; **Fig. 11.20**), spindle-cell or sarcomatoid tumours[225,226] and clear-cell carcinomas. The last must be distinguished from metastatic renal

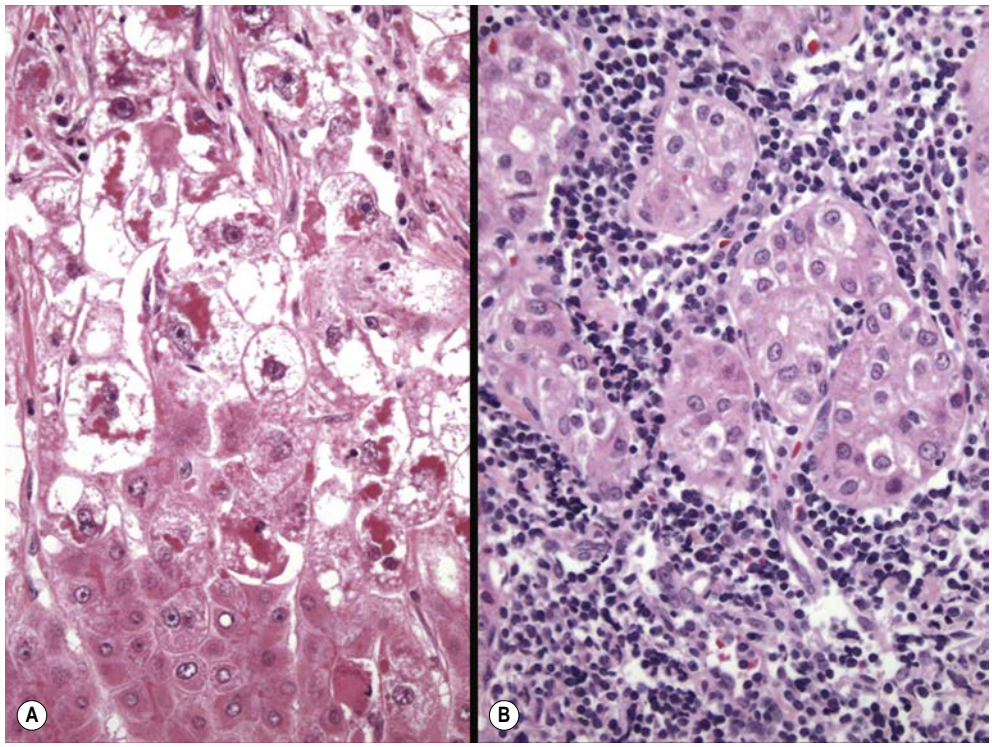

Fig. 11.19 Hepatocellular carcinoma (HCC). A: Steatohepatitic variant of HCC. The neoplastic cells are ballooned and show oedematous and rarefied cytoplasm, focal intratumour inflammation and numerous Mallory–Denk bodies. **B:** Lymphoepithelioma variant of HCC. This tumour elicits prominent lymphocytic infiltrates, chiefly T cells. (Operative specimens, H&E.)

adenocarcinoma and PAX-8 immunostain nuclear positivity is helpful evidence of the latter.[227,228a,228b] Fine-needle aspiration yields diagnostic material in a high proportion of patients.[229–232] Histological grading of HCC from 1 to 4 is based on nuclear features, with grade 1 HCC resembling normal hepatocytes and grade 2 showing prominent nucleoli, hyperchromatism and nuclear membrane irregularities.[233] Grades 3 and 4 show progressively greater nuclear pleomorphism, the latter featuring anaplastic and giant tumour cells (**Fig. 11.20**). The World Health Organisation (WHO) incorporates the growth pattern as well as the cytologic nuclear features into the grading distinctions between well-differentiated (thin trabeculae), moderately differentiated (thick trabeculae) and poorly differentiated (sheet-like) HCCs.[234] The grade of differentiation has significant prognostic implications. An example is the moderately differentiated HCC termed *macrotrabecular-massive* (trabeculae composed of >6 neoplastic hepatocytes) which has a more aggressive course, possibly because of enhanced angiogenesis resulting from overexpression of Ang2 (angiopoietin 2) and VEGF (vascular endothelial growth factor) A.[235]

When there is doubt about the hepatocellular origin of a carcinoma, further evidence can sometimes be gained from the characteristics of the tumour cells. In HCC these often contain fat and glycogen, and may also contain α_1-antitrypsin globules, even in patients without genetic α_1-antitrypsin deficiency. Mallory–Denk bodies may be found in the cytoplasm of the tumour cells,[236] particularly in the SH-HCC variant.[215] In those cases due to chronic hepatitis B, sometimes the hepatitis B core and surface antigens are demonstrable immunohistochemically in neoplastic cells.[237] Evidence of hepatocellular origin is also provided when immunohistochemical stains of paraffin sections

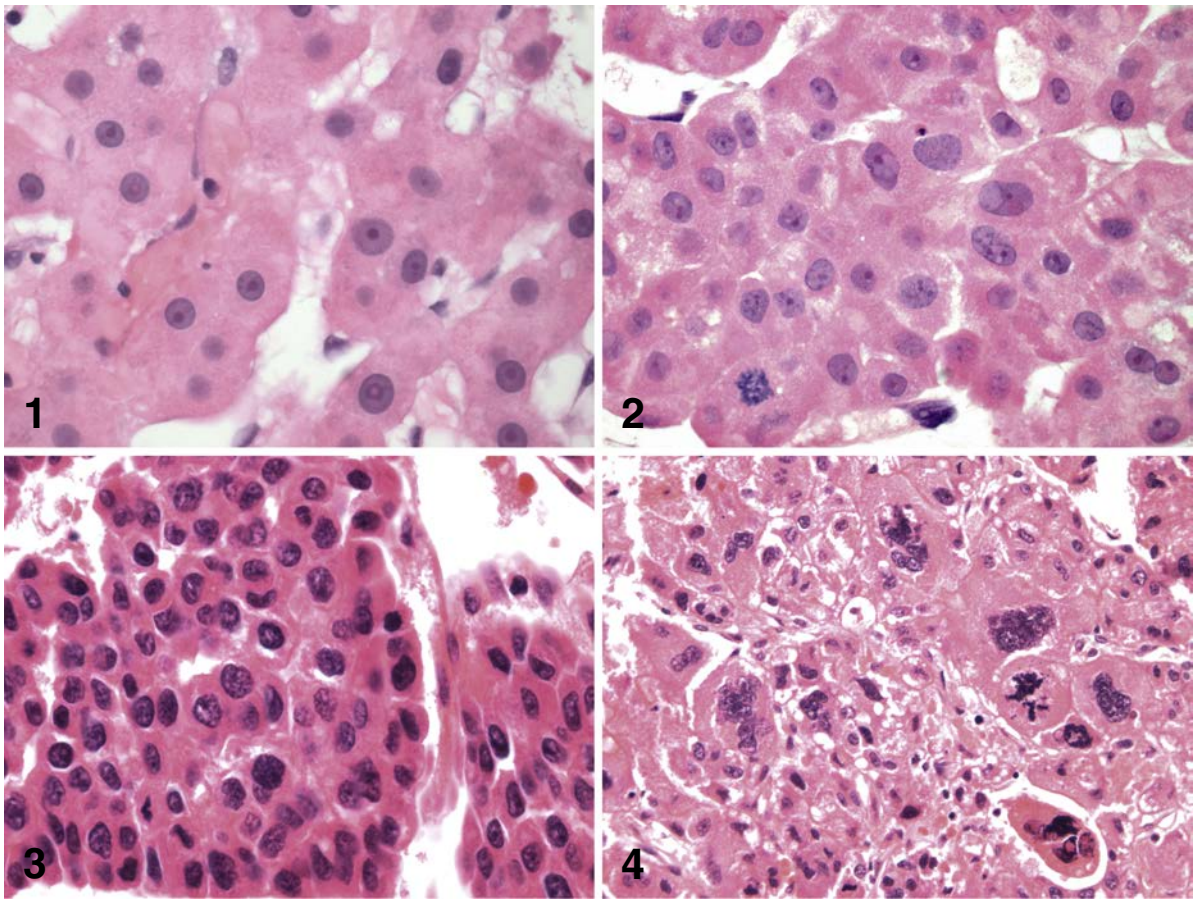

Fig. 11.20 Hepatocellular carcinoma: cytological grading. Grades 1–4 are illustrated in the respective panels. Grade 1 (well-differentiated) tumours have small, round nuclei similar to those of normal and cirrhotic liver. Grades 2 and upwards show progressive alterations in nuclear contour, chromatin coarseness and chromaticity. Grade 4 shows marked anaplasia with giant, multinucleated tumour cells and atypical mitotic figures. (Needle biopsies, H&E.)

are positive for albumin, fibrinogen, liver-cell cytokeratins (8 and 18), α_1-antitrypsin or α_1-antichymotrypsin.[238–242]

There are several possible immunohistochemical strategies for confirming the diagnosis of HCC (**Fig. 11.21** and **Table 11.4**). A useful approach is to begin with the quartet of cytokeratin 7, cytokeratin 20, arginase-1 (and/or Hep Par 1 (hepatocyte)) and bile-salt export pump (BSEP). HCC typically is negative for both cytokeratin 7 and cytokeratin 20,[243] while arginase-1 and Hep Par 1 stain normal and malignant hepatocytes (and, rarely, several extrahepatic tumours[244,245]). Hep Par 1 staining may be only patchy in needle biopsies of HCC or negative with more poorly differentiated tumours, liabilities which can be surmounted using other immunostains, such as the immunostain for BSEP, which has excellent specificity and sensitivity[245] (**Fig. 11.22**). With poorly differentiated HCC, BSEP positivity of canalicular/apical structures may be very focal, which emphasizes the need for extra diligence by the pathologist in reviewing this immunostain in such cases. CD10 and polyclonal carcinoembryonic antigen (pCEA) show similar results to BSEP but are less sensitive.[246,247] Alpha-fetoprotein is

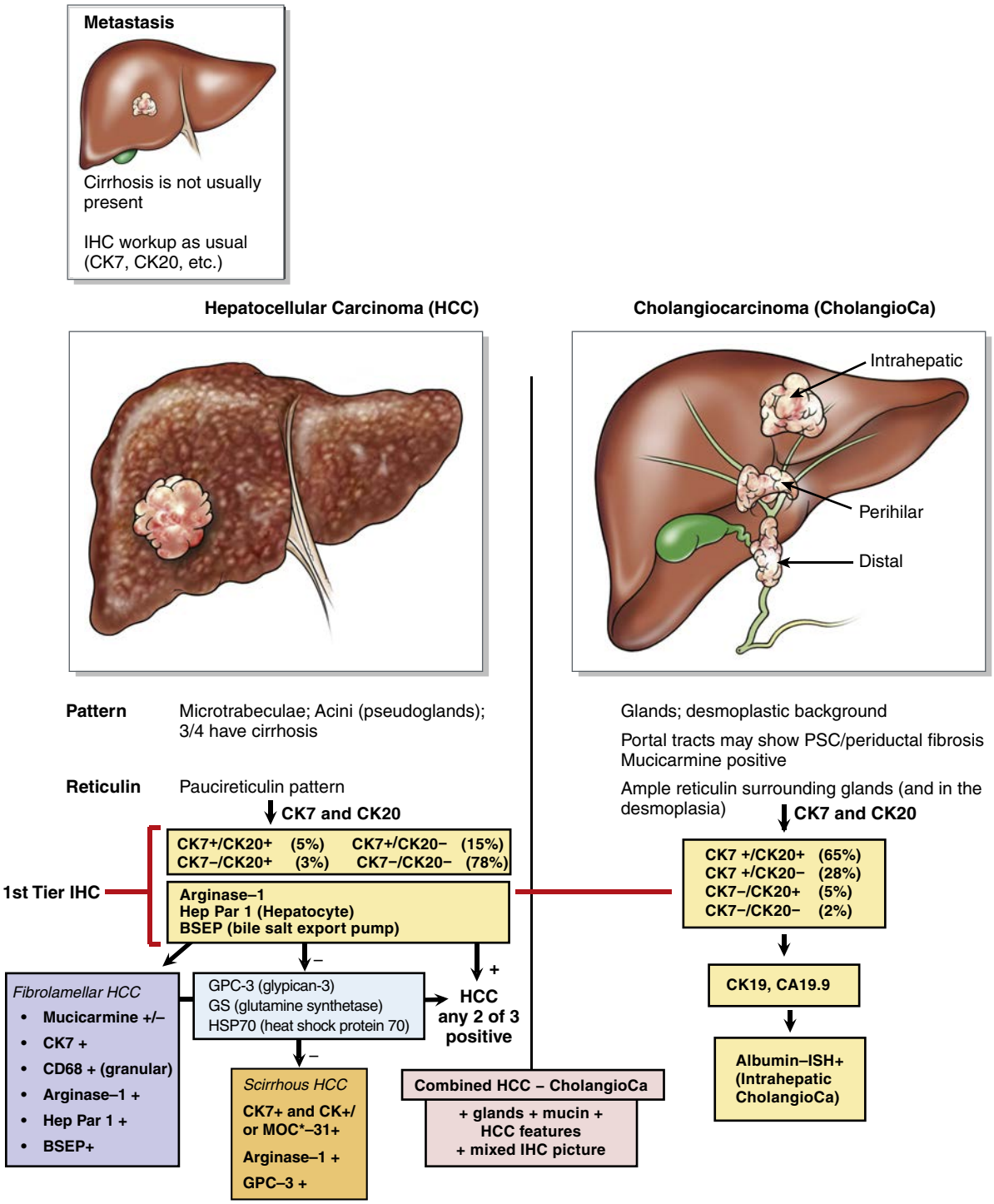

Fig. 11.21 Immunohistochemical (IHC) workup of primary malignant liver tumours. The standard diagnostic workup (in yellow) of these primary hepatic malignancies utilises cytokeratins 7 (CK7) and 20 (CK20) and arginase-1 (or Hep Par 1 +/– BSEP). Other second- and third-tier immunostains may be necessary for less-than-well-differentiated tumours, for histological variants and for tumours with mixed features (shown in other colours). Percentages for CK7 and CK20 are cited in Omata and colleagues.[214] MOC-31 = Epithelial cell adhesion molecule (EPCAM). BSEP, Bile-salt export pump; HCC, hepatocellular carcinoma; pCEA, polyclonal carcinoembryonic antigen; PSC, primary sclerosing cholangitis.

Table 11.4 Immunohistochemical stains in the evaluation of hepatic tumours.

Tumour	Recommended immunostain(s)
Hepatocellular carcinoma	Arginase-1 Hep Par 1 (hepatocyte) Bile-salt export pump (BSEP)* Cytokeratin 7/20 pair (–/– staining)† GPC-3/GS/HSP70 trio‡
Hepatoblastoma	α-Fetoprotein (AFP) Hep Par 1 (hepatocyte) Polyclonal carcinoembryonic antigen
Cholangiocarcinoma	Cytokeratin 7/19 pair (+/+ staining) Cytokeratin 7/20 pair (+/+ staining)† Carbohydrate antigen 19-9 (CA19-9)
Angiomyolipoma	HMB-45
Epithelioid	CD34
Haemangioendothelioma	CD31 Factor VIII
Metastatic carcinoma	
Neuroendocrine	Chromogranin Synaptophysin Neuron-specific enolase
Pancreas	Cytokeratin 7/20 pair (+/+ staining)†
Colorectal	Cytokeratin 7/20 pair (–/+ staining)†
Breast	Cytokeratin 7/20 pair (+/– staining)†
Lung (non-small cell)	Cytokeratin 7/20 pair (+/– staining)†

*Staining is canalicular or apical.
†See reference 242.
‡HSP70, heat shock protein 70; GPC-3, glypican-3; GS, glutamine synthetase. At least two of the three should be positive (see reference 252).

an unreliable immunostain for HCC,[248] in contrast to hepatoblastoma, where most cases stain positively. Arginase-1 immunostain has the highest sensitivity for HCC.[245] It should be kept in mind, however, that in a recent study[249] as many as 10% of well-differentiated HCCs showed negative staining for arginase-1; such cases rely on other appropriate immunostains[250,251] as well as routine morphology (such as bile production by the tumour) for a definitive diagnosis. The immunostain for thyroid transcription factor-1, often used in the diagnosis of lung carcinomas, showed positive cytoplasmic (not nuclear) staining in the majority of HCCs in one study,[252] which may be helpful in specific diagnostic settings.

The trio of GPC-3, GS and heat shock protein 70 immunostains is another valuable combination for the diagnosis of HCC, particularly when any two of the three are positive.[253] GPC-3, a cell-surface heparan sulphate proteoglycan, usually shows cytoplasmic positivity in the tumour cells, but may also be membranous or canalicular. It has particular value in staining poorly differentiated HCCs that are negative with Hep Par 1 and/or arginase-1, and is also applicable to fine-needle aspiration specimens.[254] In hepatitis C-related

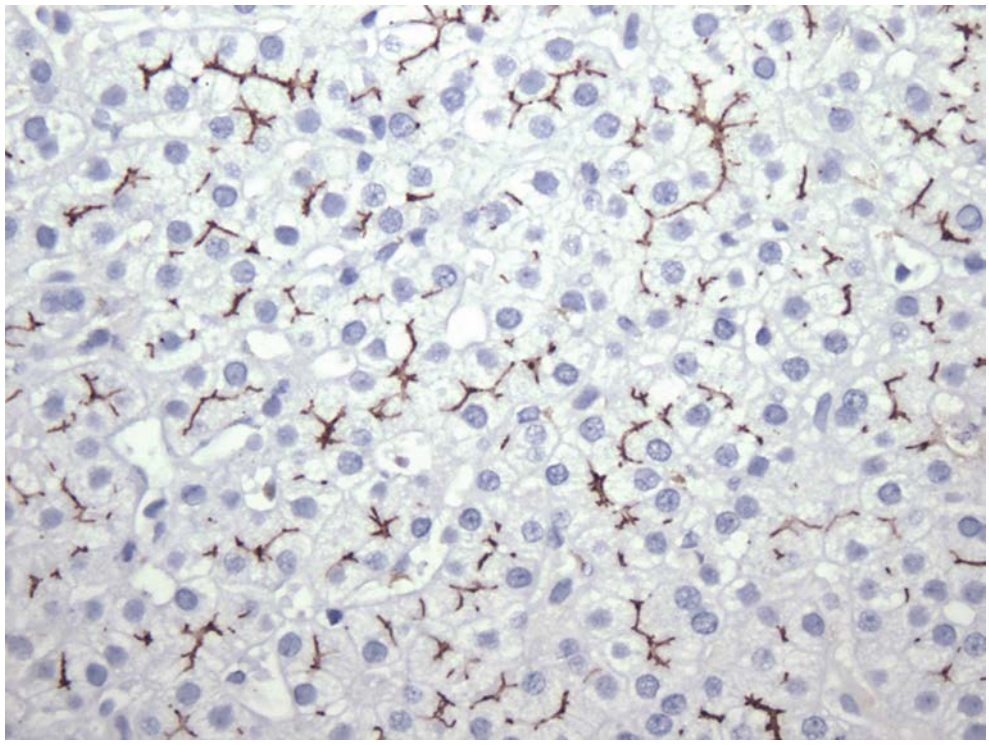

Fig. 11.22 Immunohistochemical demonstration of bile canalicular structures using an immunostain for bile-salt export pump. The branching spaces are bile canaliculi, here outlined by immunohistochemical staining. Similar canalicular (or 'membranous'/'apical') staining is seen in hepatocellular carcinoma. (Operative specimen, specific immunoperoxidase.)

cirrhosis, nodules with high-grade necroinflammatory activity have been noted to show strong GPC-3 positivity.[255,256] Positive GPC-3 staining may also be seen in certain germ cell tumours,[257] ovarian clear-cell carcinoma,[258] squamous cell carcinoma of the lung,[259] some gastrointestinal tract carcinomas and acinar pancreatic carcinoma.[260] GS staining in non-neoplastic liver is restricted to the cytoplasm of perivenular hepatocytes, while HCC shows diffuse strong lesional staining.[142,253] Heat shock protein 70 shows focal nucleocytoplasmic positivity in HCC.[142] This panel of three immunostains also helps distinguish dysplastic lesions from HCC.[253]

Certain tumours show mixed features of both hepatocellular and cholangiocytic differentiation or are phenotypically "intermediate" or "stem/progenitor" in appearance. A recent international consensus report provides guidelines for these complex cases.[260a] "Stemness" or progenitor/stem-cell features can be further evaluated with hepatic progenitor/stem-cell immunohistochemical markers, including neural cell adhesion molecule (NCAM), epithelial cell adhesion molecule (EpCAM), cytokeratin 7 (CK7) and CK19, c-KIT (CD117) and CD133.[261–263] CK19 positivity has been associated with HCC invasiveness.[264]

Fibrolamellar carcinoma

This tumour usually develops in non-cirrhotic liver in older children and adults and carries a better prognosis (because of its resectability[265] and absence of cirrhosis[266]) than typical HCC.[267–276] The lesions are solitary or multiple and occasionally resemble FNH macroscopically in having a central fibrous scar.[277] The unique histological features distinguish this tumour from routine HCC. Fibrous lamellae are arranged in parallel separate groups of large, densely eosinophilic tumour cells[269,278] which produce transforming growth factor-β[279] (**Fig. 11.23**). The eosinophilia is due to the presence of abundant

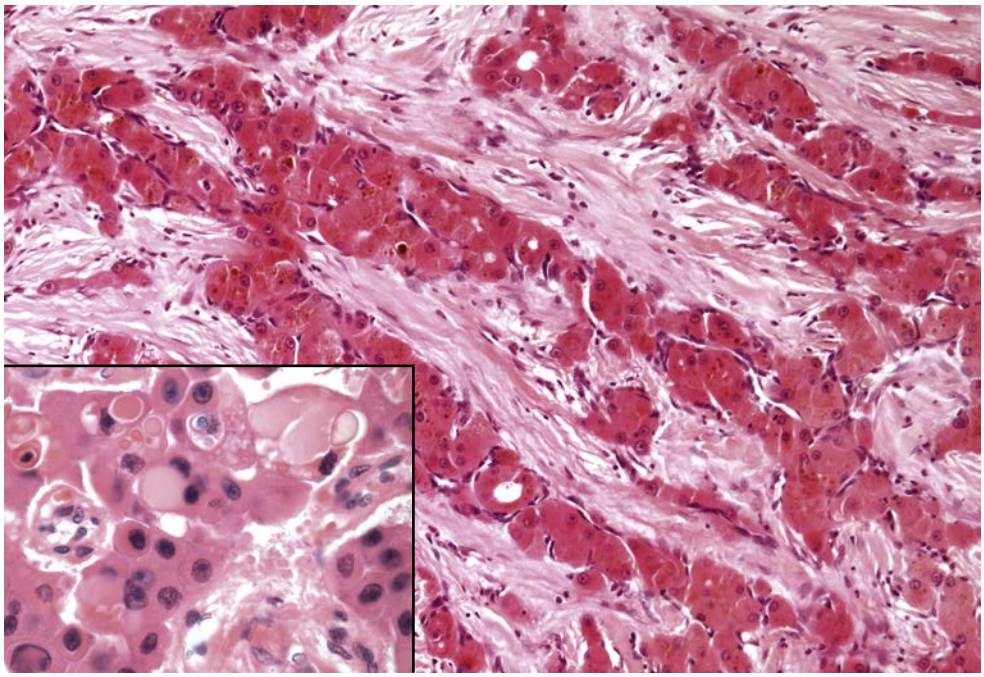

Fig. 11.23 Hepatocellular carcinoma: fibrolamellar type. Groups of large, eosinophilic tumour cells are surrounded by fibrous septa in parallel arrays. (Needle biopsy, H&E.) Inset: Tumour cells contain 'pale bodies' (top centre). Several hyaline bodies are also evident in tumour cells at top, left of centre. (Explant liver, H&E.)

mitochondria.[268,280] Tumour cells commonly contain eosinophilic, diastase–PAS-negative globules which stain immunohistochemically for C-reactive protein, fibrinogen and α_1-antitrypsin, as well as cytoplasmic 'pale bodies' which are reactive for fibrinogen.[269] Tumour cells are positive with Hep Par 1, arginase-1, and cytokeratin 7 immunostains.[270,271] Additional features include bile production (as in other forms of HCC), copper and copper-associated protein within tumour cells[281,282] and stainable CEA in bile canaliculi.[283] Some fibrolamellar carcinomas have neuroendocrine features,[284,285] mucicarmine-positive pseudoglands[286] or show features of both fibrolamellar and typical HCC.[287] CD68 immunostain shows positive granular stippling of the lysosomes and endosomes within the neoplastic cells[288] (see the 'Cytopathological diagnosis' section; **Fig. 11.49**). Rarely, fibrolamellar HCC is associated with Fanconi's anaemia.[289] The majority of patients with fibrolamellar HCC have a mutation on chromosome 19 that results in a *DNAJB1–PRKACA* fusion gene and production of a chimeric protein with preserved protein kinase A function. Genetically engineered mice with this fusion gene also develop fibrolamellar liver tumours.[272] The molecular driver for fibrolamellar HCC in 80%–100% of patients appears to be this fusion gene.[273] In addition to the distinctive morphology of this form of HCC and immunostain positivity for CK7 and CD68, additional documentation of the fibrolamellar subtype can be achieved with fluorescence *in situ* hybridisation (FISH) for the *PRKACA* rearrangement.[290] In rare instances, fibrolamellar HCC is part of the Carney complex, in which skin pigmentation lesions, nodular masses of thyroid and adrenal glands and cardiac myxomas are variably admixed. Such cases show an alternative gene mutation (loss) affecting *PRKAR1A*, the gene regulating activation of protein kinase A.[291]

Bile-duct carcinoma (cholangiocarcinoma)

Carcinoma of the bile ducts can arise anywhere between the papilla of Vater and the smaller branches of the biliary tree within the liver. It is not usually associated with cirrhosis. Three

types are recognised by anatomical site of involvement, including distal bile duct, perihilar bile ducts (so-called Klatskin tumour[292]) and intrahepatic bile ducts.[293,294] The incidence of intrahepatic cholangiocarcinoma has been rising worldwide in recent decades.[295,296] The most common known predisposing factors to bile-duct cancer are infestation with hepatobiliary flukes (*Opisthorchis viverrini* and *Clonorchis sinensis*), primary sclerosing cholangitis[297] and congenital cystic lesions of the biliary tree.[298,299] Of these, Caroli's disease and choledochal cysts are important precursors, but carcinoma may also arise in von Meyenburg complexes (bile-duct microhamartomas)[300] and in congenital hepatic fibrosis.[301] Development of carcinoma in bile-duct adenoma is also reported.[302] In Japan, hepatitis B and C virus infections have been suggested as a risk factor[303] and intrahepatic cholangiocarcinoma is a known consequence of hepatolithiasis.[304,305] Current genomic and molecular studies of cholangiocarcinoma[306] have demonstrated the importance of mutational events affecting inflammatory,[307,308] oncogene (*KRAS* and *BRAF* especially[309,310]) and metabolic (e.g. *isocitrate dehydrogenase 1* and *2* genes[311]) pathways in the aetiopathogenesis of this tumour (**Table 11.2**). Specification of the genomic derangement by molecular profiling studies is important in targeted tumour therapy.[310] Lesions suspicious for cholangiocarcinoma are often evaluated by endoscopic cholangiography with retrieval of brushings and cytopathological specimens. FISH evaluation for polysomy in tandem with routine morphology and, where indicated, immunohistochemistry, should be considered in the pathological workup of these frequently difficult diagnostic lesions.

Microscopically, bile-duct carcinomas are mucin-secreting adenocarcinomas with a reactive, desmoplastic fibrous stroma (**Fig. 11.24**). A fairly uniform gland size (medium to

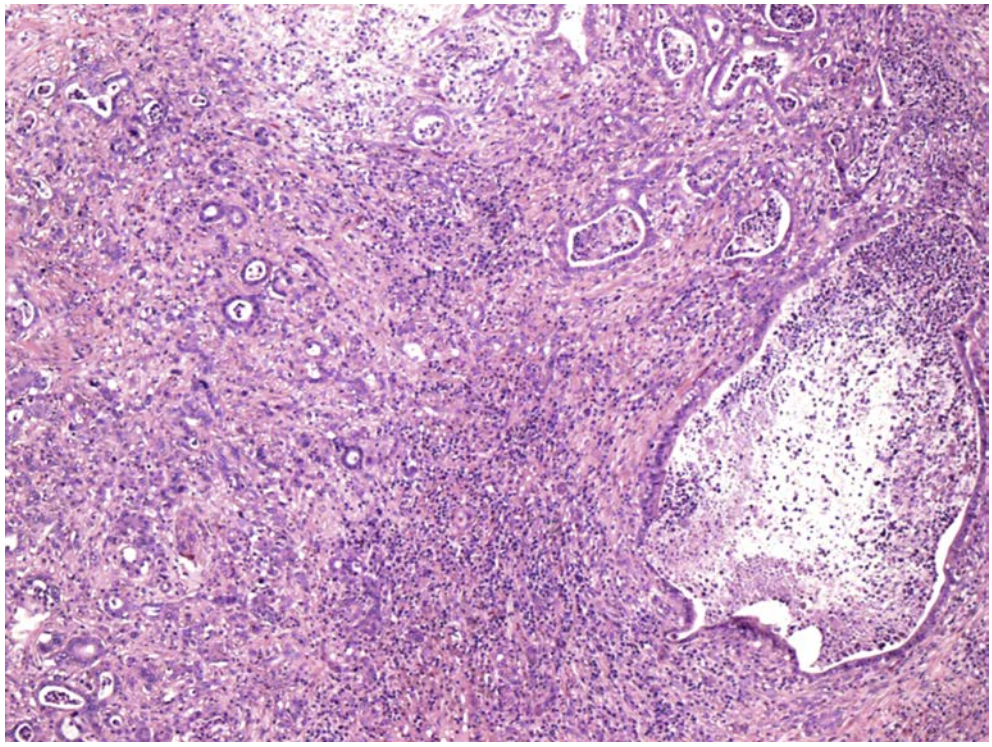

Fig. 11.24 Bile-duct carcinoma (cholangiocarcinoma). There are many medium- and small-sized neoplastic glands invading the desmoplastic fibrous stroma. The neoplastic cells of cholangiocarcinoma are typically cuboidal to low columnar. The adjacent native bile duct is dilated and contains neutrophils due to associated cholangitis. The appearances are different from those of the hepatocellular carcinoma of adenoid pattern shown in **Fig. 11.15**. (Operative specimen, H&E.)

small) is often maintained within these tumours, in comparison with the wide size variations seen in glands of metastatic pancreatic carcinoma. The **cholangiolocellular carcinoma** subtype shows interanastomosing antler-like ductular structures composed of small cuboidal cells with scant cytoplasm that appear to evolve from progenitor cells in the region of the canal of Hering (**Fig. 11.25**).[312,313] The tumour cells are cuboidal or columnar and may assume a papillary pattern. Adenosquamous, squamous, mucinous,[314] clear-cell[315] and anaplastic histological types are less common.[316] A small number of intrahepatic cholangiocarcinomas are predominantly mucinous and show CK7 positivity, CK20 and CDX2 negativity and microsatellite stability (unlike colorectal adenocarcinomas).[317] Rarely, a **lymphoepithelioma-like cholangiocarcinoma** is encountered which, like its hepatocellular counterpart, has a significant lymphocytic component admixed with the neoplastic glands.[220] Cholangiocarcinomas often show intraneural and perineural invasion. The presence of free stromal mucin, small groups and isolated tumour cells in fibrous stroma, and the concurrence of apparently normal epithelium and abnormal tumour cells within a duct-like structure all help to distinguish cholangiocarcinoma from metastatic tumour.[318] Cholangiocarcinoma must be distinguished from the acinar type of HCC, a distinction usually made with confidence on the basis of mucin or bile secretion, respectively. In difficult cases positive staining for epithelial membrane antigen,[319] tissue polypeptide antigen,[320] biliary cytokeratins[239] (7 and 19), Lewis(x) and Lewis(y) blood group-related antigens[321] and α-amylase[322] helps to exclude HCC. In the uncommon tumour which shows combined hepatocellular–cholangiocarcinoma, cytokeratins 7 and 19 and epithelial membrane antigen immunostaining is positive in the cholangiocellular component.[323,324] Other rare mixed tumours show sarcomatoid[325] or fibrolamellar regions.[326] Bile-duct tumours are very occasionally of neuroendocrine type, with characteristic neurosecretory granules in their cytoplasm. A rare, neuroendocrine-like microtubular/microcystic variant of intrahepatic cholangiocarcinoma with blastemal-like regions ('cholangioblastic' cholangiocarcinoma) showing CK7, CK19, chromogranin, synaptophysin and inhibin A positivity has

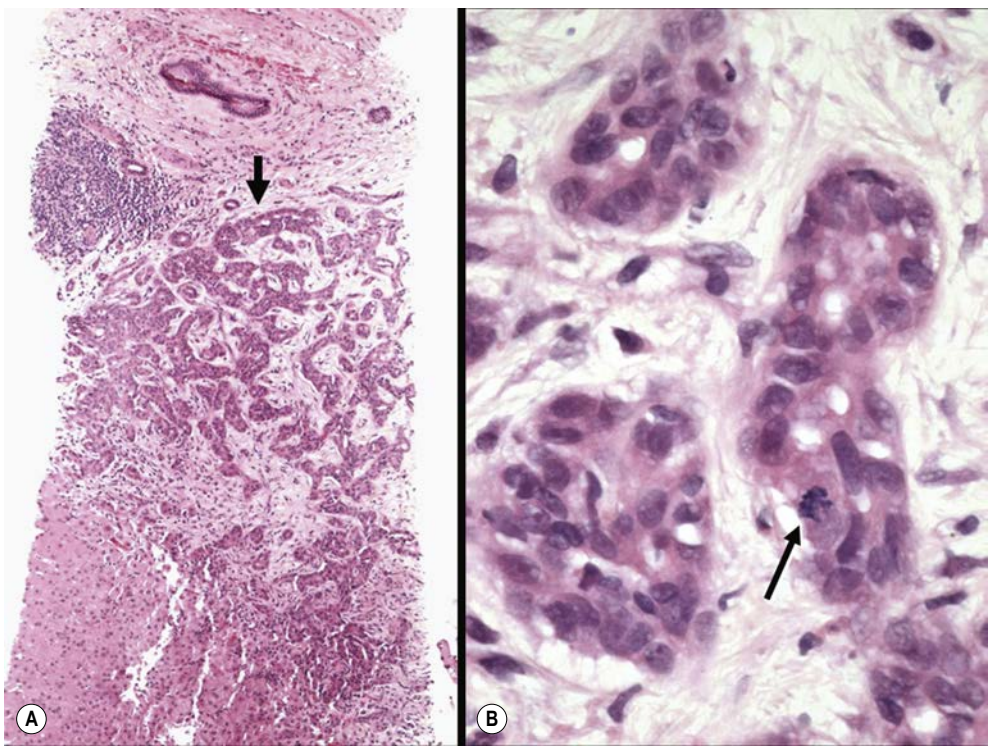

Fig. 11.25 Cholangiolocellular carcinoma. A: These tumours typically grow from peripheral regions (arrow), the sites of hepatic progenitor cells. The neoplastic glands grow with an 'antler-like', branching pattern, infiltrating the adjacent liver tissue within a fibrous stroma. **B:** Nuclear atypia and mitotic figures (arrow) are present. (Needle biopsy, H&E).

been described in young women.[327] The differential diagnosis of bile-duct cancer includes epithelioid haemangioendothelioma and metastatic adenocarcinoma. No specific immunohistochemical stain is currently available for definitive identification of cholangiocarcinoma. Positivity for cytokeratins 7 and 20 (or cytokeratin 7 alone), for cytokeratin 19 and for CA19.9 is supportive evidence for cholangiocarcinoma, but such positivity does not, for example, exclude metastasis to the liver of a primary pancreatic adenocarcinoma. For intrahepatic cholangiocarcinoma, however, *in situ* hybridisation for albumin (*albumin ISH*) has proven to be of great diagnostic value and has become a mainstay in the pathological workup of carcinomas in the liver of uncertain primary site.[328] This value resides in the presumed mutual derivation of hepatocytes and the neoplastic cells of intrahepatic cholangiocarcinoma from the same or similar stem cell(s) found in the primitive liver bud. Perihilar and distal bile-duct cholangiocarcinoma are negative with albumin ISH.

Cystadenocarcinomas are rare malignant tumours which sometimes develop from benign cystadenomas.[106,108] Although these have been considered distinct from the more aggressive carcinomas arising from pre-existing congenital cystic lesions,[329] occasional tumours with features of cystadenocarcinoma develop in fibropolycystic disease.[330]

Angiosarcoma

This uncommon, highly malignant tumour forms multiple or, less often, solitary haemorrhagic masses. Predisposing factors include treatment with arsenic,[331] injection of the radioactive contrast medium Thorotrast[332–334] and industrial exposure to vinyl chloride.[335] Other postulated factors include copper-containing vineyard sprays,[336] steroid hormones,[337–339] phenelzine[340] and urethane.[341] Positive staining of tumour cells for factor VIII–related antigen and other endothelial markers is evidence of their endothelial origin.[342,343] Their growth is characteristically along sinusoids and around surviving hyperplastic hepatocytes (**Fig. 11.26**). The presence of the latter may lead to confusion with HCC, with which angiosarcoma, however, occasionally coexists. Infiltration of sinusoids beyond the main tumour mass makes the outlines of the tumour indistinct. Both cavernous and solid areas may be present. Other features include islands of haemopoietic cells, and areas of thrombosis and infarction.

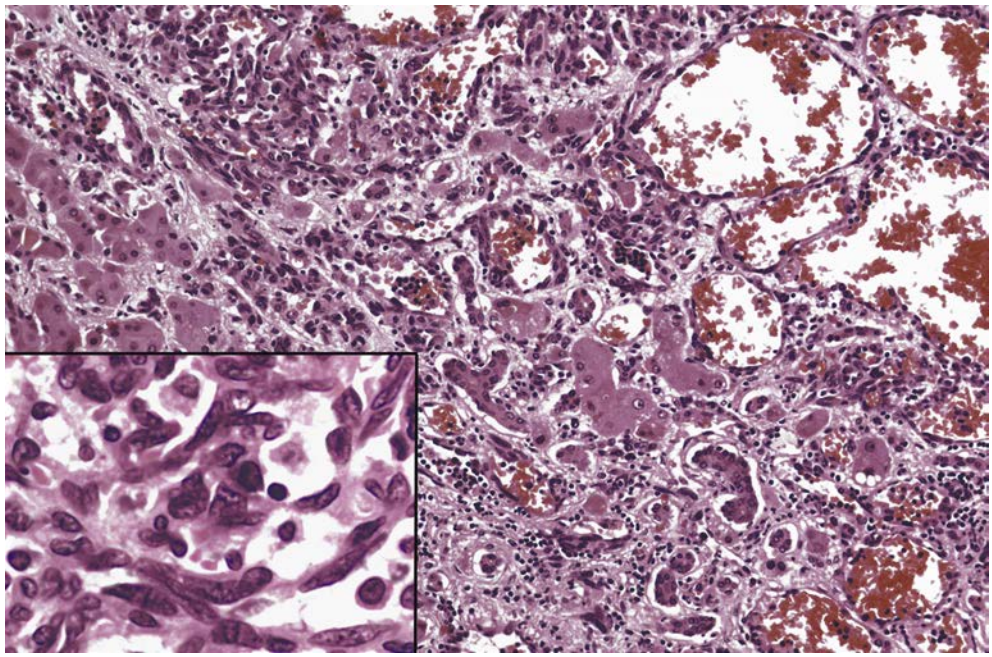

Fig. 11.26 Angiosarcoma. Elongated tumour cells surround islands of hepatocytes (centre) in this highly vascular tumour. Inset: Pleomorphic endothelial cells line the vascular spaces. (Operative specimen, H&E.)

The non-neoplastic liver tissue is usually not cirrhotic, but may show fibrosis and other changes attributable to the aforementioned predisposing factors, including deposits of refractile Thorotrast granules in macrophages. Features seen irrespective of the cause include focal dilatation of sinusoids, hyperplasia of hepatocytes, sinusoid-lining cells and perisinusoidal cells, and increased perisinusoidal reticulin.[344] These changes may precede the development of the tumour.[345]

Epithelioid haemangioendothelioma

This endothelial tumour of soft tissues or the lung (intravascular bronchioloalveolar tumour) may uncommonly present as a primary liver tumour. In the liver it is seen in patients from the second to eighth decades of life, with women more commonly affected.[346–348] Its prognosis varies very widely: some patients survive for decades while others die within months of diagnosis.[349] Histologically, it may be confused with adenocarcinoma or with veno-occlusive disease. Its causes are unknown, but a relationship to oral contraceptive use has been postulated.[350]

The lesion consists of proliferated endothelial cells with pleomorphic nuclei, arranged in clusters or singly, some of them with rounded lumens (**Fig. 11.27**). The lumens may be mistaken for lipid or for mucin droplets in a signet-ring cell adenocarcinoma. Two types of tumour cells have been described,[346,348] dendritic and epithelioid, the latter giving rise to the adenocarcinoma-like appearance. The tumour cells should be positive on immunostaining for one or more endothelial markers (CD34, CD31, factor VIII[348]). CD34 immunostaining is more sensitive than factor VIII.[351] Further evidence of vascular differentiation is seen ultrastructurally where Weibel–Palade bodies in tumour cells and a tumour tissue component of pericytes have been noted.[352] High cellularity is a predictor of unfavourable prognosis, whereas nuclear pleomorphism and mitotic count are not.[348]

Vascular occlusion by dense fibrous tissue containing tumour cells, a characteristic feature, is seen in both portal and hepatic vein branches. This is best seen with connective tissue stains. The problem of confusion with veno-occlusive disease or even steatohepatitis is compounded by the fact that the tumour sometimes has a zonal distribution, affecting perivenular regions of each lobule in a more or less regular fashion (**Fig. 11.28**).

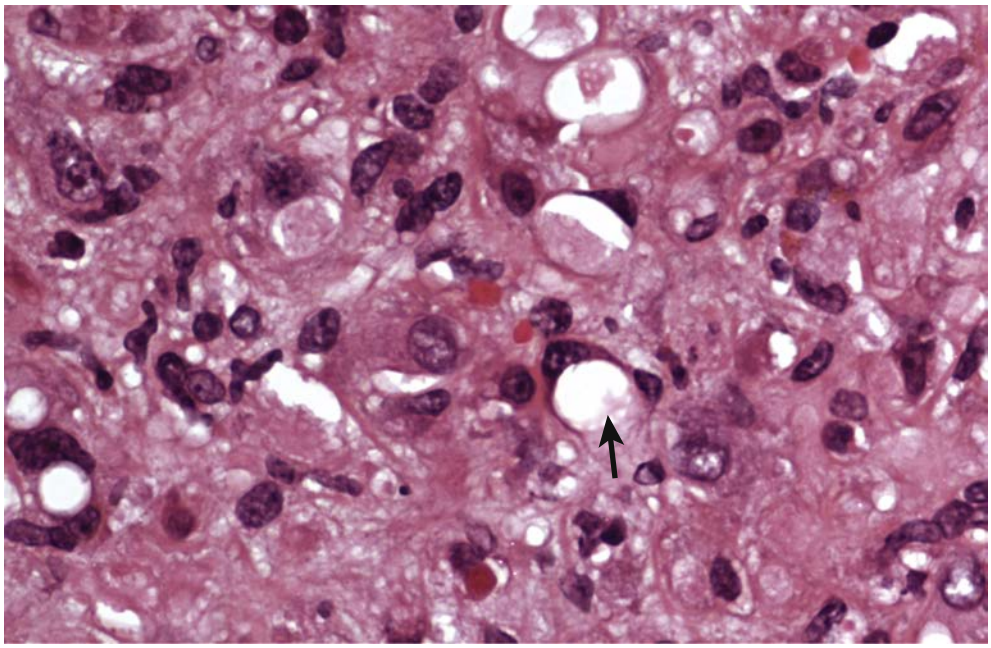

Fig. 11.27 Epithelioid haemangioendothelioma. Individual tumour cells and small groups are set in a dense fibrous stroma. Some of the tumour cells have formed vascular lumens (arrow). (Operative specimen, H&E.)

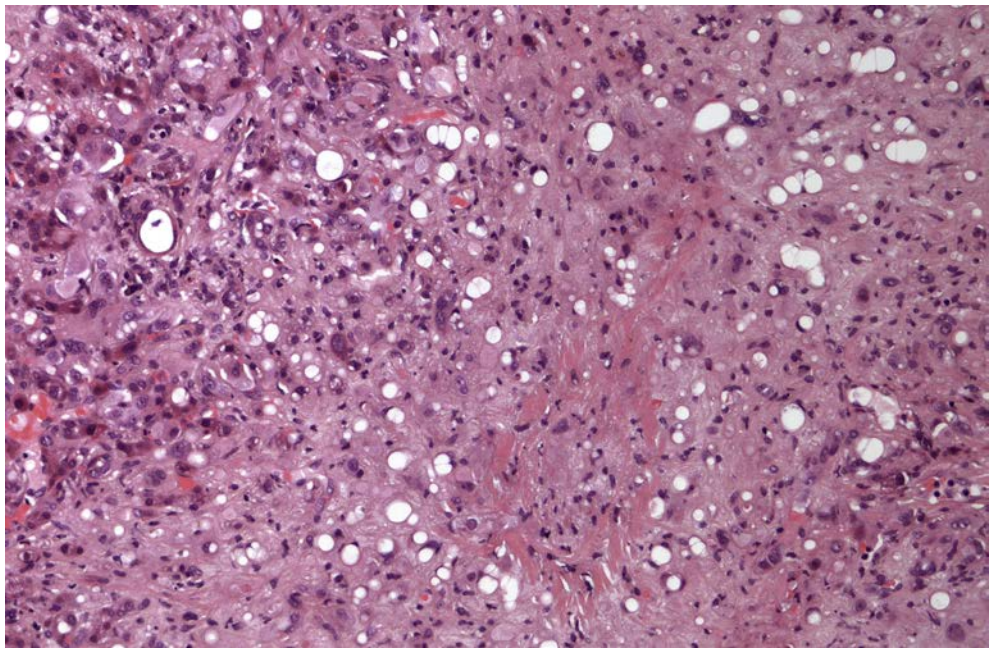

Fig. 11.28 Epithelioid haemangioendothelioma. In this example the tumour has a zonal distribution, mimicking the fibrosis of venous outflow obstruction. The tumour stroma is predominantly seen in the perivenular and mid-zonal regions, while surviving periportal hepatocytes and ductular reaction are evident at left and at lower right. (Operative specimen, H&E.)

Extrahepatic malignancy and the liver

Patients with extrahepatic tumour may have biochemical evidence of hepatic dysfunction in the absence of liver metastases, particularly when the tumour is a renal adenocarcinoma. Liver biopsies in such patients have shown Kupffer-cell proliferation, hepatocellular swelling, focal necrosis, fatty change and mild inflammation.[353,354] Granulomas are occasionally found and there may be cholestasis, especially in Hodgkin's disease (discussed later).

Metastatic tumour

Blind percutaneous needle biopsy may reveal metastatic tumour, but the yield of correct diagnoses is increased if the needle is guided by means of an imaging method. Multiple punctures may be needed to sample the tumour. Guided fine-needle aspiration is a helpful diagnostic procedure,[355,356] and cytological examination of aspiration fluid and touch preparations of biopsy specimens increase the yield of positive results.[357] Step sections of biopsy specimens should be examined if tumour is suspected clinically but initial sections are negative. The primary site of a tumour can sometimes be determined histologically. Some metastases, notably from renal adenocarcinoma, can mimic HCC, and metastatic tumour may invade liver-cell plates, giving a false impression of primary carcinoma arising within them (**Fig. 11.29**). Many metastatic tumours are associated with extensive desmoplastic fibrous stroma. Immunohistochemistry is usually required for confirmation of the primary site in such cases. Before concluding that the neoplasm is metastatic from a distant site, the abundant stroma should serve to remind the pathologist to at least consider (and dismiss, when not relevant) the possibility of a primary scirrhous HCC (**see Fig. 11.17**). There are also primary tumours of the stomach, and less often of the ovary, oesophagus,

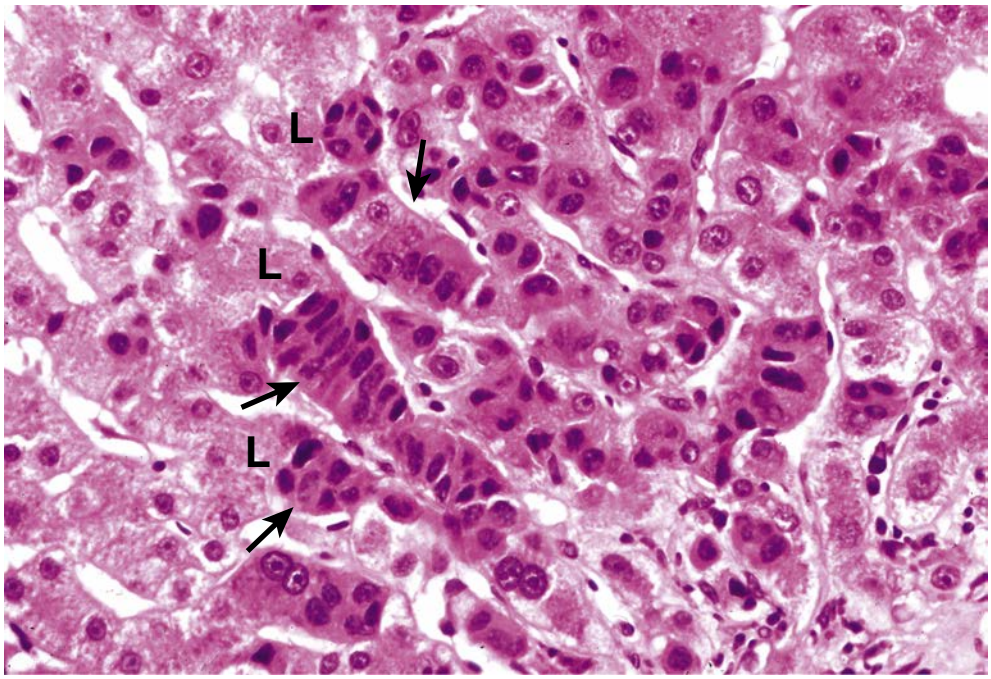

Fig. 11.29 Metastatic tumour. Cells of a carcinoid tumour (arrows) have invaded liver-cell plates (L), giving a false impression of origin from the latter. (Needle biopsy, H&E.)

lung, gallbladder, pancreas, bladder, uterus and colon that may closely resemble HCC in both their primary sites and metastatic foci. These **hepatoid carcinomas** may produce bile and stain positively with a variety of the immunohistochemical markers used to diagnose HCC.[208]

Biopsy specimens from the vicinity of a metastasis typically show portal oedema, ductular reaction and infiltration by neutrophils, as well as focal sinusoidal dilatation[358] (**see Fig. 1.7**). The ductular structures sometimes have abnormal epithelium with atypical, hyperchromatic nuclei. The portal changes are reminiscent of those seen in biliary obstruction.

Lymphomas and leukaemias

Hodgkin's disease

Liver biopsy plays an important part in staging; wedge biopsies are more likely than multiple needle biopsies to reveal deposits, and either may be positive in spite of normal macroscopic appearances of the liver at laparotomy.[359] Negative biopsy does not rule out liver involvement. Hepatic involvement by Hodgkin's disease is usually associated with splenic involvement.[360] Step sections of initially negative small biopsies should be examined because the infiltrates of Hodgkin's disease are unevenly distributed and may be sparse. Correct diagnosis of an infiltrate may be difficult because Reed–Sternberg cells are often very scanty, so that the correct diagnosis must be suspected on the basis of other features. These include an abnormal population of cells with deeply stained angular nuclei or vesicular nuclei with prominent nucleoli (**Fig. 11.30**), irregular infiltration beyond portal tracts with destruction of hepatocytes and abundant reticulin fibres. There is a variable component of reactive lymphoid cells, eosinophils and histiocytes. The differential diagnosis of Hodgkin's disease in the liver includes reactive infiltrates and other lymphomas, especially of the T-cell type.

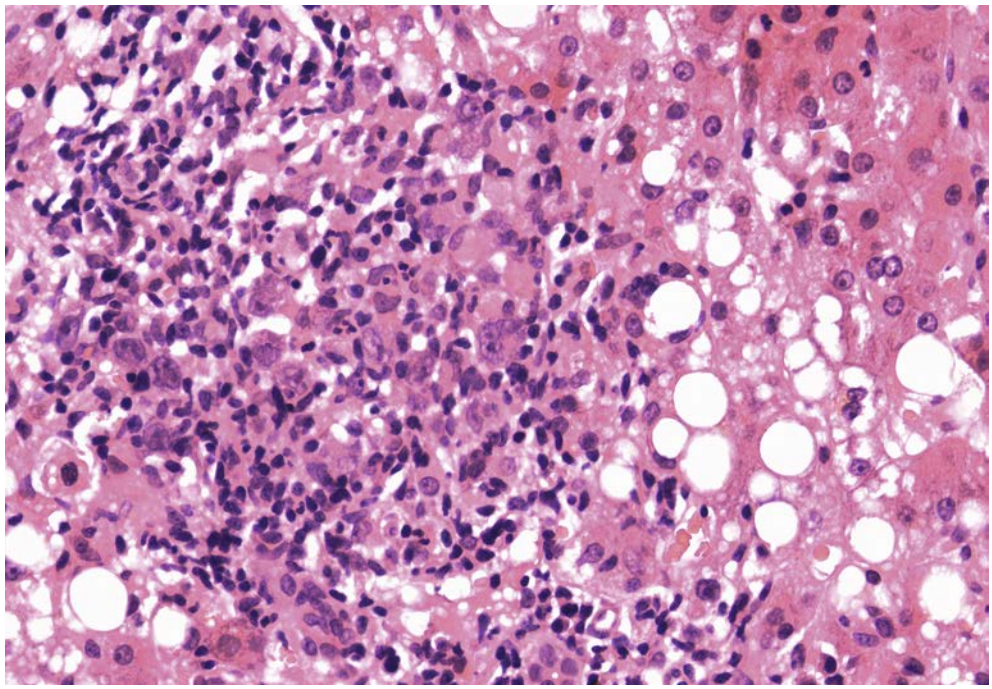

Fig. 11.30 Hodgkin's disease. The portal infiltrate is composed of a variety of cells, including large tumour cells with angular, hyperchromatic nuclei. (Needle biopsy, H&E.)

A variety of non-specific changes may be seen in parts of the liver adjacent to the malignant deposits. Even in the absence of malignant deposits there may be lobular lymphoid aggregates with some degree of cellular atypia[361] or epithelioid-cell granulomas.[362] Sinusoidal dilatation with or without Hodgkin's infiltrates in the liver has been reported, most often in patients with general symptoms.[363] The lesion is most severe in acinar zones 2 and 3. **Cholestasis** in Hodgkin's disease is uncommon, seen in the absence of hepatic infiltration in some patients[364] but more often as a feature of advanced disease. In some cases cholestasis is explained by destruction of interlobular bile ducts (ductopenia) related directly to the malignant infiltrates or resembling that seen in ductopenic rejection after liver transplantation.[365,366]

Non-Hodgkin's lymphoma and other haemopoietic malignancies

Non-Hodgkin's lymphomas primarily involve portal tracts, but may spread to periportal parenchyma and sinusoids.[367] Predominantly sinusoidal infiltration is also recognised.[368] Tumour deposits and fibrosis may cause portal hypertension[369] and, rarely, massive infiltration presents clinically as liver failure.[370] Vasculitis is another rare presentation. Malignant infiltrates can usually be distinguished from inflammatory ones by their dense and homogeneous appearance, and by the total or near-total involvement of portal tracts. Substantial apoptosis and necrotic debris may also be seen in lymphoma (**Fig. 11.31**) but are not characteristic of benign infiltrates. Immunohistochemical stains are important in establishing the type of lymphoma.[371] The disease is usually systemic, with involvement of lymphoid tissues as well as liver. **Primary hepatic lymphoma** is quite rare, representing less than 1% of extranodal lymphomas.[372–374] Both B-cell lymphomas, including **mucosa-associated lymphoid tissue (MALT) lymphoma**,[375,376] and T-cell lymphomas are seen with B-cell tumours predominating.[373,377] Many B-cell lymphomas (splenic marginal B-cell lymphoma, follicular and diffuse large B-cell lymphoma[378]) and proliferative diseases (mixed cryoglobulinaemia, monoclonal gammopathy) and, rarely, T-cell lymphoma[379] are associated with underlying chronic hepatitis C virus infection.[380] Chronic hepatitis C with

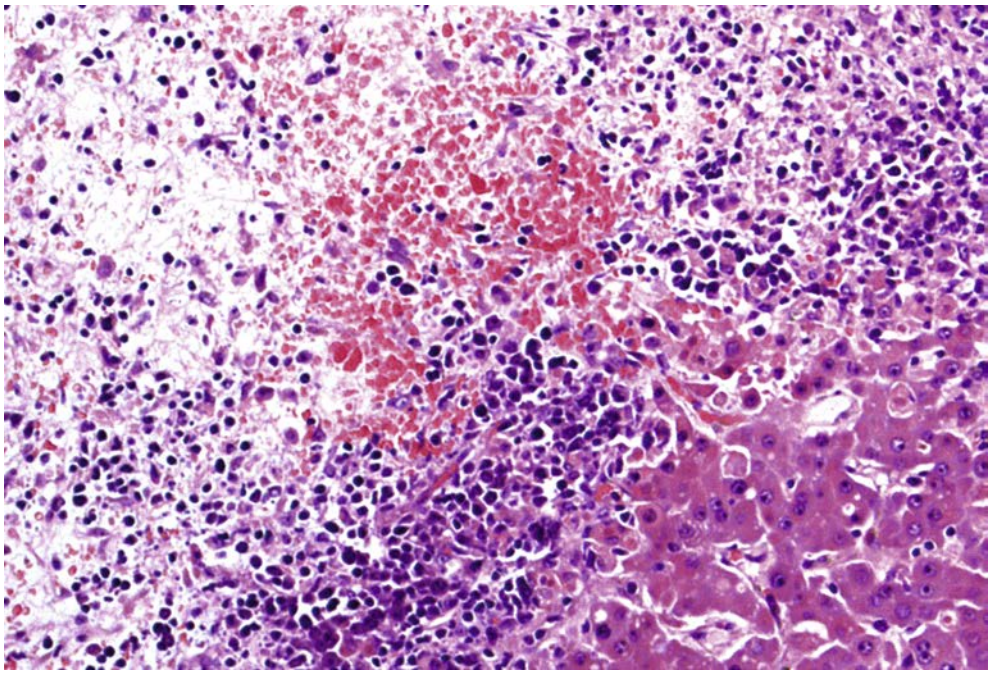

Fig. 11.31 Non-Hodgkin's lymphoma. Tumour cells are seen irregularly infiltrating the adjacent periportal liver parenchyma. Extensive tumour cell necrosis is apparent. (Wedge biopsy, H&E.)

concomitant B-cell lymphoma and HCC may also occur.[381] **Peripheral γ-δ**[382] and rare **α-β**[383] **T-cell lymphomas** with hepatic sinusoidal infiltration and splenic involvement have also been described. Primary hepatic lymphomas present as solitary or multiple masses, as diffuse hepatic involvement with hepatomegaly, or as liver failure with elevated serum lactate dehydrogenase activity.[373]

The liver may be diffusely or focally infiltrated in **multiple myeloma**.[384] Solitary primary hepatic plasmacytoma has also been reported.[385] In **macroglobulinaemia**,[386] mononuclear cells, some with pyroninophilic cytoplasm, may be found in portal tracts and sinusoids. Both diseases are sometimes complicated by amyloid deposition. **Langerhans' cell histiocytosis** involving only the liver may rarely occur.[387,388] The infiltrating Langerhans' cells, positive on immunostains for langerin, S-100 and CD1a, may invade and destroy interlobular bile ducts, leading to ductopenia, chronic cholestasis and features resembling primary sclerosing cholangitis[389] (**Ch. 13**). In **systemic mast-cell disease** with liver involvement, infiltration of portal tracts by mast cells is associated with fibrosis.[390] Parenchymal infiltrates are also seen. The infiltrating cells may be rounded, histiocyte-like or spindle-shaped. Their nature may not be suspected on routine stains of paraffin sections; plastic sections or special mast-cell stains make the diagnosis clear.

The infiltrates of various **leukaemias** are often seen in the liver and are frequently accompanied by steatosis and/or fibrosis.[391] Schwartz and Shamsuddin[392] reported hepatic involvement in nearly all cases of **chronic lymphocytic leukaemia** examined, and noted marked widening of portal tracts with portal–portal linking and a variable degree of fibrosis. In **hairy cell leukaemia** the hepatic sinusoids are infiltrated by the leukaemic cells, often identifiable by the halo-like, clear cytoplasm around rounded or indented nuclei.[393] However, these are not always present.[394] Another histological characteristic is the formation of angiomatous lesions, in which vascular channels in portal tracts or acini are lined by leukaemic cells rather than by endothelium. Endothelial disruption with communication of sinusoids with the perisinusoidal space of Disse is seen on electron microscopy.[395] Staining for tartrate-resistant acid phosphatase in paraffin sections has sometimes been helpful in diagnosis.[396]

Neoplasms and nodules in children

Benign lesions

Rarely, **liver-cell adenoma** may develop spontaneously in children with no underlying disease or exposure to hormones.[397] In this age group it has also been associated with Fanconi's anaemia,[40] type I glycogen storage disease, Hurler's disease, severe combined immunodeficiency,[398] the antiepileptic agent oxcarbazepine[399] and mutations in hepatocyte nuclear factor-1α (see the discussion on liver-cell adenoma in adults later). The vast majority pursue a benign course, but transformation to HCC after many years of observation has been reported.[400] The identification of **FNH** in infancy and in adulthood has been taken as additional evidence that it is a tumour-like malformation rather than a true neoplasm. **NRH** is unusual in childhood. It occurs as early as 7 months of age and shows the same histological features as in adults.[401] Hepatosplenomegaly and portal hypertension may be present and in some patients there is a history of prior chemotherapy or anticonvulsant medication.

Mesenchymal hamartoma

This is an uncommon lesion of infancy and childhood, rarely seen in older patients. Loose, oedematous connective tissue rich in blood vessels contains lymphangioma-like cystic spaces, bile ducts and hepatocytes[402,403] (**Fig. 11.32**). Haemopoietic cells are often present. The edge of the lesion is irregular, gradually merging with adjacent normal liver. In adults the bile-duct elements may be difficult to find and the collagenous stroma is densely hyalinised.[404] An undifferentiated embryonal sarcoma arising in mesenchymal hamartoma has been reported.[405] These tumours are associated with

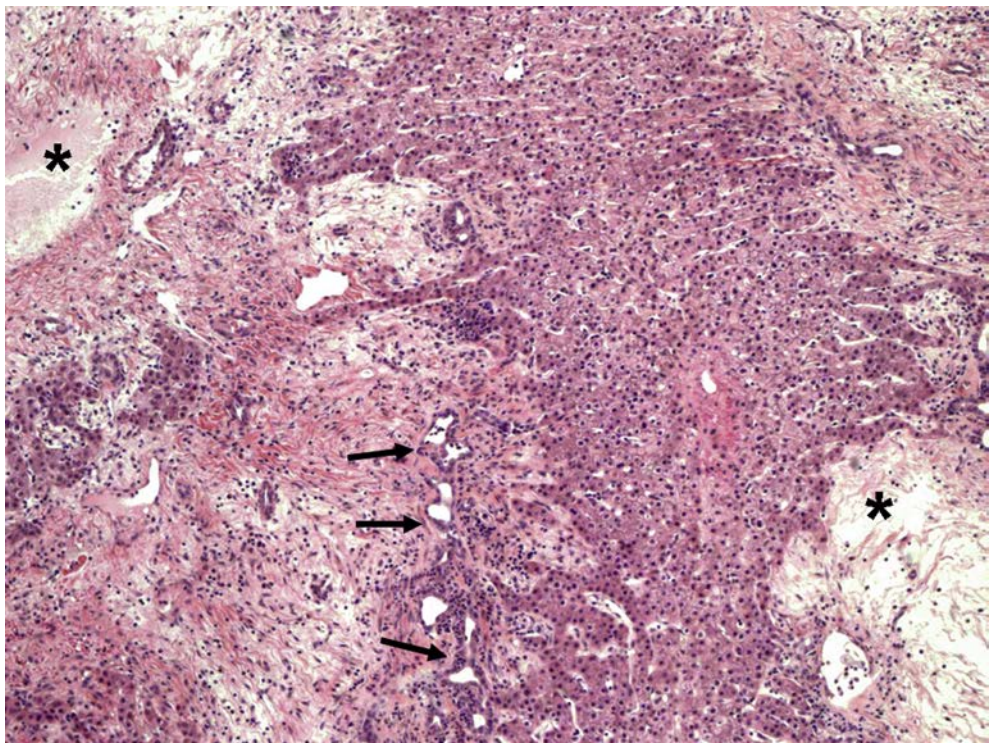

Fig. 11.32 Mesenchymal hamartoma. The combination of tissues seen in this benign neoplasm includes loose connective tissue with cystically dilated lymphatics (asterisks), bile ductular structures (arrows) and geographic islands of liver parenchyma (centre). (Operative specimen, H&E.)

Beckwith–Wiedemann syndrome, placental mesenchymal dysplasia, aberrant activation of the chromosome 19 microRNA cluster[406] (C19MC) located on chromosome 19q13.4 (with resulting dysregulation of microRNA profiles) and, recently, with mutations in the *DICER* gene on chromosome 14 (a gene known to be associated with a variety of cystic neoplasms and multinodular goitre).[6]

Infantile haemangioendothelioma

This solitary or multicentric tumour is composed of capillary-like vascular channels lined by plump endothelium (**Fig. 11.33**), which with time undergo progressive maturation, scarring and eventual involution.[115,407,408] Central portions of the tumour may show increased fibrous stroma, and thrombosis and dystrophic calcification are sometimes present. The margin of the tumour often merges into adjacent liver parenchyma. Dehner and Ishak[407] described type I tumours with cytologically bland endothelium and type II tumours capable of aggressive behaviour and metastasis, with atypical, hyperchromatic endothelium and intravascular budding. The latter are now considered angiosarcomas.[409] Most cases present in the first 6 months of life with hepatomegaly, abdominal mass or diffuse abdominal enlargement.[115,407,410] There may be high-output cardiac failure due to shunting through the tumour, liver failure or tumour rupture. The possibility that some of these tumours will pursue a malignant course should be kept in mind in evaluating the histopathology of individual cases.

Malignant lesions

Hepatoblastoma

Hepatoblastoma is the most common liver tumour in childhood,[411] usually presenting at less than 2 years of age. The prognosis depends on surgical resectability and histological type. These tumours are usually solitary and histologically classified into two essential

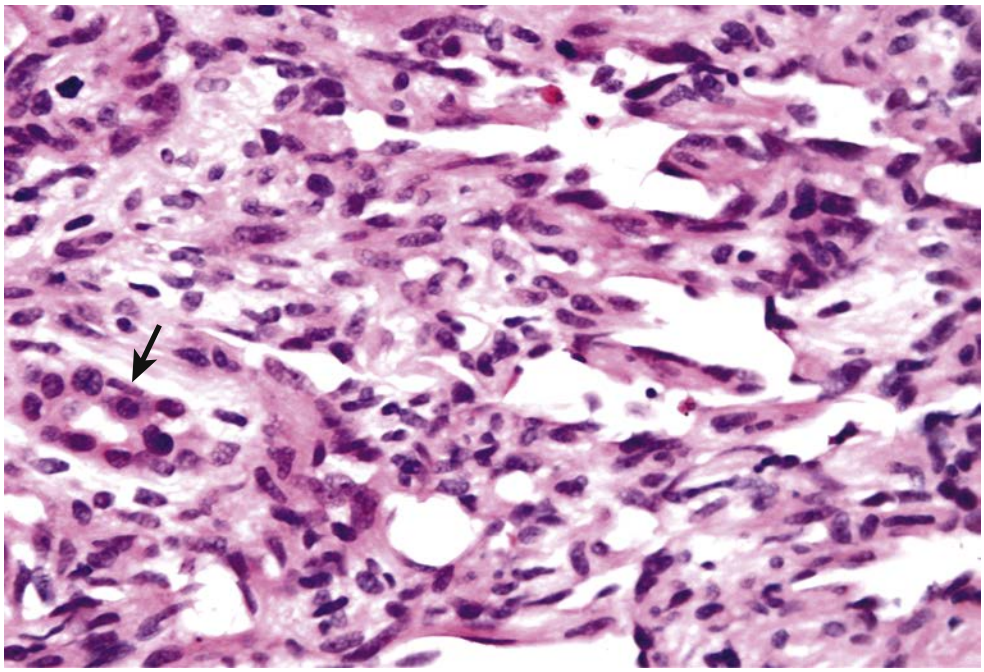

Fig. 11.33 Infantile haemangioendothelioma. The tumour is composed of vascular channels lined by plump endothelium. Entrapped bile ducts are often present (arrow). (Operative specimen, H&E.)

Table 11.5 Histological classification of hepatoblastoma*

Epithelial variants
Pure fetal with low mitotic activity
Fetal, mitotically active
Pleomorphic, poorly differentiated
Embryonal
Small-cell undifferentiated
INI-1-negative†
INI-1-positive
Epithelial mixed (any/all above)
Cholangioblastic
Epithelial macrotrabecular pattern
Mixed epithelial and mesenchymal
Without teratoid features
With teratoid features
Hepatocellular carcinoma
Classic hepatocellular carcinoma (HCC)
Fibrolamellar HCC
Hepatocellular neoplasm NOS‡

*Recommended classification[416] from the COG (Children's Oncology Group)
†INI-1 (integrase interactor 1, involved in chromatin remodelling and cellular transcriptional regulation).
‡Indicates provisional entity. Tumours previously designated as transitional liver-cell tumours may be included in this category.

categories, **epithelial** and **mixed epithelial–mesenchymal,** with a variety of histologic subtypes therein (**Table 11.5**).[412–416]

The epithelial type consists of fetal or embryonal liver cells, or both. Fetal cells somewhat resemble adult hepatocytes in appearance but are smaller (**Fig. 11.34**). The fat and glycogen content in some fetal cells gives them a pale appearance, thereby rendering a 'light-and-dark' pattern to fetal areas at low magnification. These areas are also characterised by foci of extramedullary haemopoiesis and an absence of mitoses. By histological pattern, the purely fetal type has the best prognosis.[417] Embryonal cells have less cytoplasm, higher nucleus-to-cytoplasm ratios, higher cell proliferative indices,[418] poorly defined cellular margins and mitotic activity (**Fig. 11.35**). They may form rosettes, acini or tubules. Squamous differentiation may be present in epithelial hepatoblastomas. Gonzalez-Crussi et al.[419] described a **'macrotrabecular'** pattern reminiscent of HCC but containing fetal or embryonal cells. Mixed epithelial–mesenchymal hepatoblastomas, now referred to as 'teratoid hepatoblastoma',[416] contain mesenchymal elements such as osteoid and cartilage in addition to epithelium. Staining for α-fetoprotein is common in hepatoblastoma. Hep Par 1 is positive in fetal portions of hepatoblastoma (but may be negative in embryonal regions) while GPC-3 immunostain and β-catenin (nuclear positivity) are usually positive (except in the less common histological variants). Hepatoblastomas frequently demonstrate mutations of the *CTNNB1* gene which encodes β-catenin, and a subset of these tumours shows activation of yes-associated protein 1 (YAP1).[420]

Sarcoma and lymphoma

Undifferentiated sarcomas with a poor prognosis occasionally develop in the liver in children.[421] Epithelium trapped within the tumour may give rise to confusion. Light microscopic and ultrastructural features suggest malignant fibrous histiocytoma[422,423] or myoblastic differentiation.[423] Immunohistochemical results are inconsistent, with reports of staining for histiocytic markers, desmin, vimentin and even cytokeratin.[423,424] Another form of sarcoma, arising in the biliary tract, is the **embryonal rhabdomyosarcoma** or **sarcoma botryoides.**[417] The histologically distinctive **calcifying nested stromal–epithelial tumour** is a mixed stromal and epithelial low-grade malignant neoplasm with foci of calcification or ossification.[425–431] This tumour usually grows indolently and shows nests of small, round spindled and large eosinophilic epithelioid cells arranged in irregular zigzag patterns surrounded by desmoplastic stroma with interspersed bile ducts (**Fig. 11.36**). The tumour shows nuclear and cytoplasmic positivity for β-catenin and mutations in the *β-catenin* gene.[430] Exceptionally rare **primary non-Hodgkin's lymphoma** in the liver has been reported in childhood.[432]

Hepatocellular carcinoma

HCC in children resembles the adult type histologically. Cirrhosis due to tyrosinaemia type 1, BSEP deficiency (progressive familial intrahepatic cholestasis type 2), biliary atresia and prolonged total parenteral nutrition may be present, as well as other predisposing causes such as type I glycogenosis. EpCAM appears to be an important immunohistochemical

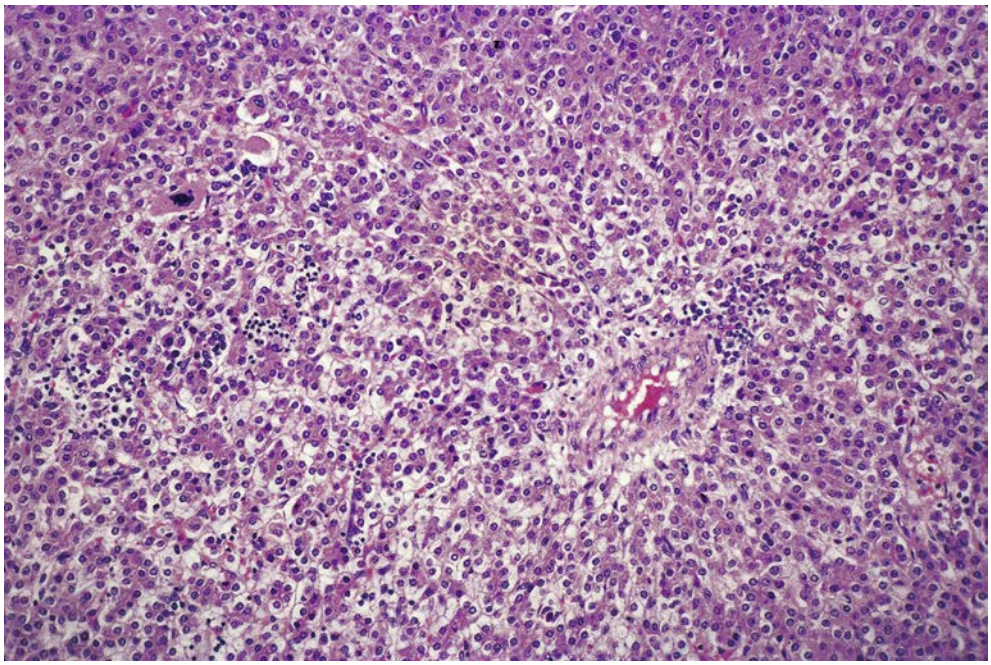

Fig. 11.34 Hepatoblastoma, fetal epithelial type. The tumour grows in cords of small hepatocytes with a 'light-and-dark' herringbone pattern due to the admixed clear (glycogenated) and eosinophilic liver cells. Foci of extramedullary haemopoiesis, including several megakaryocytes and clusters of erythrocyte precursors, are seen at upper left. (Operative specimen, H&E.)

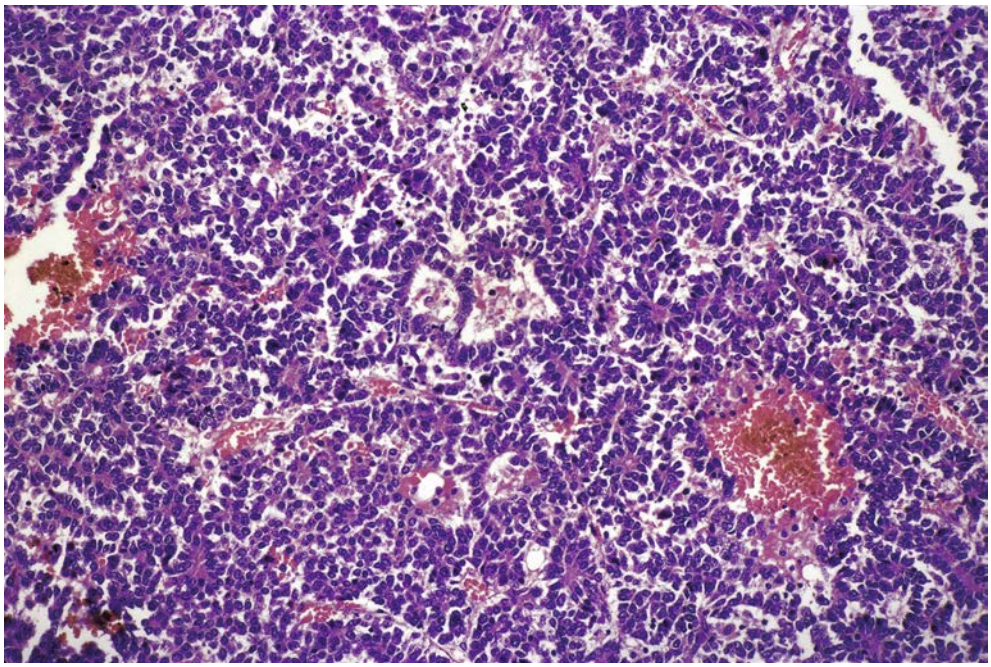

Fig. 11.35 Hepatoblastoma, embryonal epithelial type. Tumour cells grow in tubules and show an increased nucleus-to-cytoplasm ratio. Darkly stained mitotic figures can be identified in some cells. (Operative specimen, H&E.)

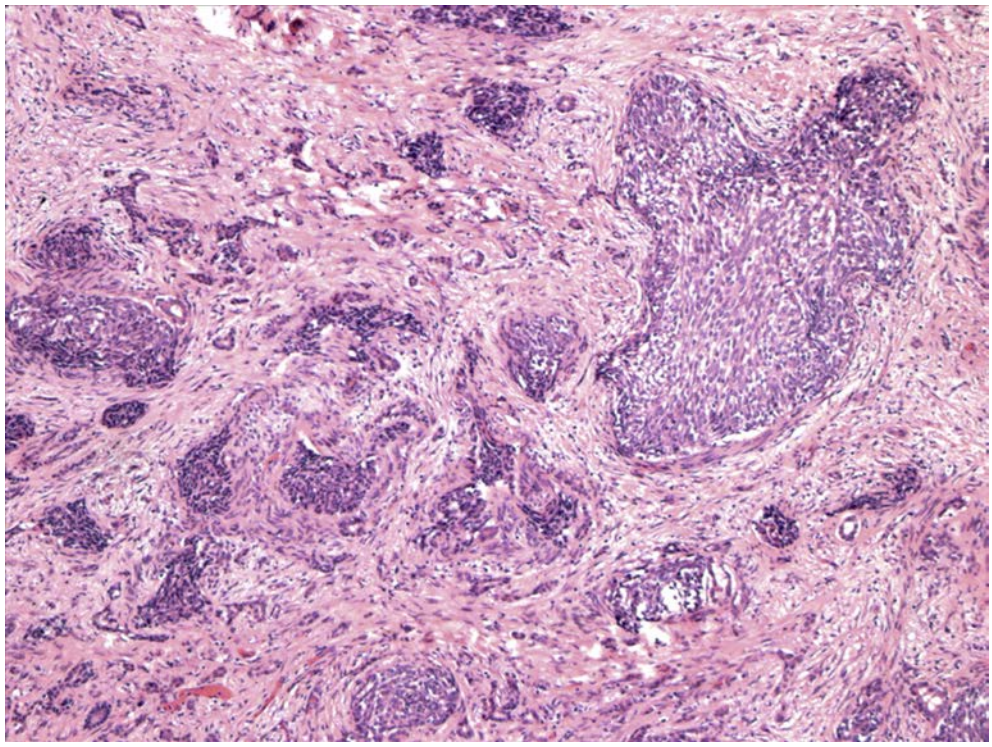

Fig. 11.36 Calcifying nested stromal–epithelial tumour. The desmoplastic stroma contains epithelial nests arranged in a 'zig-zag' pattern. The epithelium varies from larger eosinophilic cells (nest at right) to more basophilic and spindled. Bile-duct structures are in close proximity to the nests. (Partial hepatectomy, H&E.)

marker of this tumour, as well as cytokeratin 19 and GPC-3.[433] The fibrolamellar type of carcinoma has been described in older children, with better prognosis than HCC in general.[268]

Cytopathological diagnosis

FNAB is often used to investigate hepatic masses, particularly for patients with cirrhosis in whom HCC is suspected.[434] This technique may demonstrate lesional tissue as well as components of normal or non-neoplastic liver. In the latter regard, the interpreter must be familiar with the cytological appearances of normal liver, cirrhosis or dysplasia, which are discussed in the following sections. FNAB specimens may contain liver cores or liver parenchyma centrifuged into a cell block. If neoplasm is present, the concordance in interpretation between general or gastrointestinal pathologist and the cytopathologist is high.[435a] In certain tumour cases or when non-neoplastic liver is present, consultation by the cytopathologist with the general or hepatic pathologist is often helpful and important.[435b]

Normal or reactive liver

Aspirates from non-neoplastic liver will contain **normal** or **reactive hepatocytes,** which are present as single cells, clusters or two-dimensional monolayer sheets (**Fig. 11.37**). Normal hepatocytes may be arranged in trabeculae, but these should consist of three or fewer cells, without enveloping endothelium (**Fig. 11.38**). Individual hepatocytes are polygonal cells with well-defined borders and centrally placed, round nuclei which often

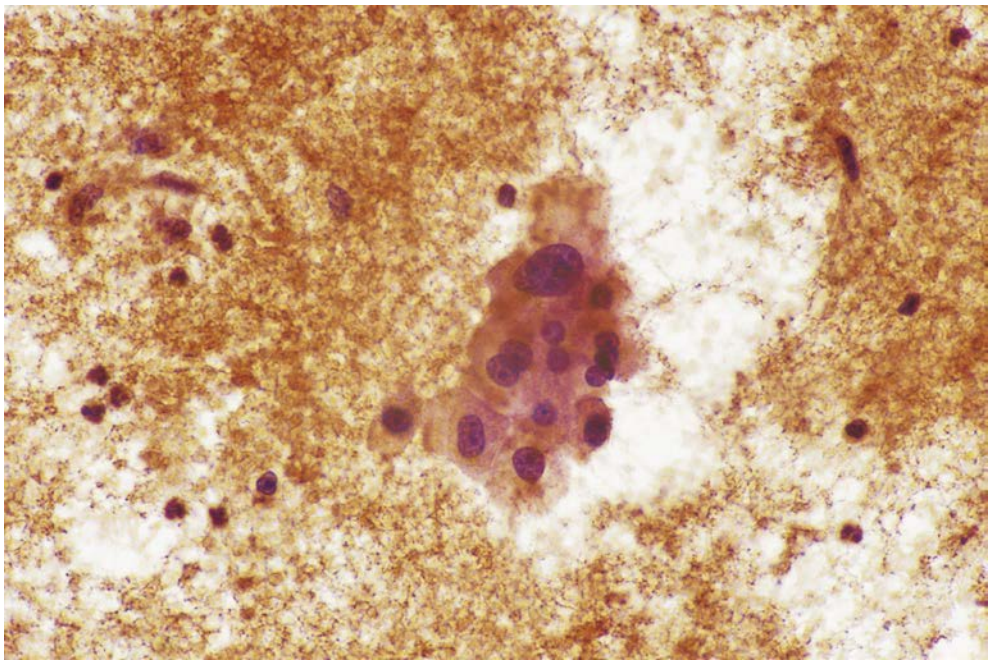

Fig. 11.37 Normal hepatocytes. A cluster of normal liver cells includes several binucleated hepatocytes and an enlarged, polyploid cell at top. Prominent nucleoli are visible. (Papanicolaou.)

have conspicuous nucleoli and occasionally show intranuclear cytoplasmic pseudoinclusions (vesicular inclusions). The latter may also be seen in HCC and are therefore not diagnostic. The nuclei of benign hepatocytes may vary considerably in size (not shape), and this is a helpful diagnostic sign which contrasts with the more monomorphic nuclei seen in HCC.[436] The appearance of pigment in the liver varies according to the staining method used.[436,437] The presence of lipofuscin in hepatocytes is indicative of a benign process.

Benign aspirates may also contain **bile-duct epithelium**, which is usually not present in specimens from liver-cell adenoma and HCC.[438] Bile-duct epithelial cells are smaller than hepatocytes, are arranged in monolayers with a 'honeycomb' glandular pattern (**Fig. 11.39**) and have eccentrically located nuclei in a pale, non-granular cytoplasm. The nuclei have fine chromatin and no prominent nucleoli. Aspirates may also contain sheets of benign **mesothelium** derived from the peritoneum (**Fig. 11.40**).

Cirrhosis and liver-cell dysplasia

Aspirates from cirrhotic liver may contain portions of connective tissue and fibroblasts (**Fig. 11.41**), bile-duct epithelium, reactive hepatocytes arranged in clusters with jagged edges (rather than smooth-edged trabeculae as in HCC) and mixed chronic inflammatory cells (mostly lymphocytes). A definitive diagnosis of cirrhosis based only on FNAB is usually not possible.[439]

Large-cell and small-cell dysplasia (**Ch. 10**) can also be identified on FNAB. The type and degree of nuclear atypia distinguish dysplastic hepatocytes from normal or reactive liver cells. In large-cell dysplasia, nuclei are enlarged, hyperchromatic and pleomorphic, with one or more prominent nucleoli (**Fig. 11.42**). Coarse nuclear chromatin and pseudoinclusions of invaginated cytoplasm are often present. The presence of cellular enlargement with ample cytoplasm maintains a relatively normal nucleus-to-cytoplasm ratio. In small-cell dysplasia this ratio is increased because the atypical nuclei are found

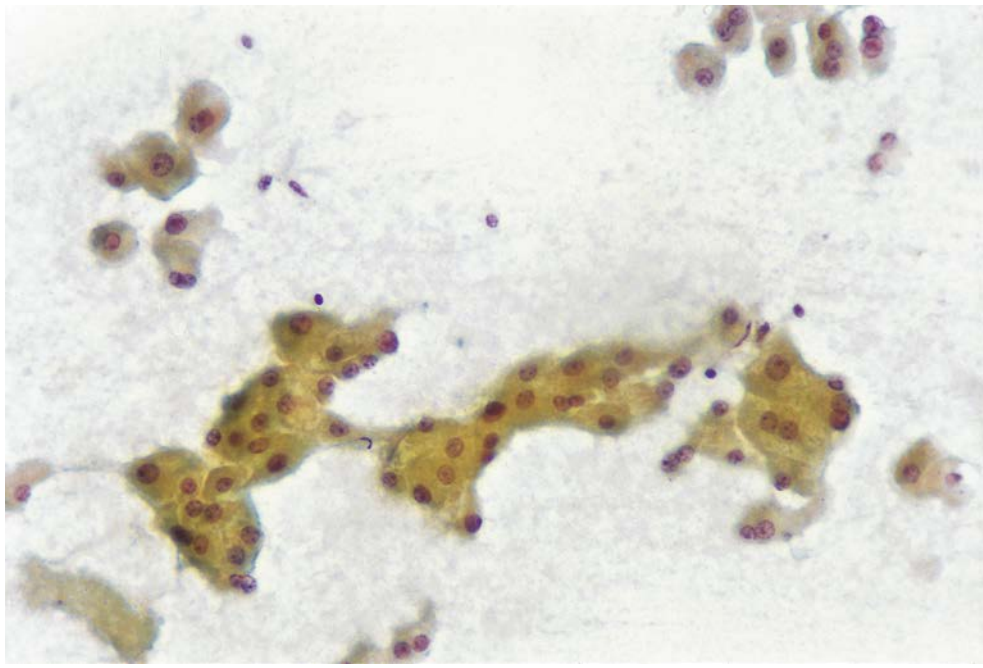

Fig. 11.38 Normal hepatocyte trabeculae. Normal trabeculae of hepatocytes on aspirate contain up to two or three cells. (Papanicolaou.)

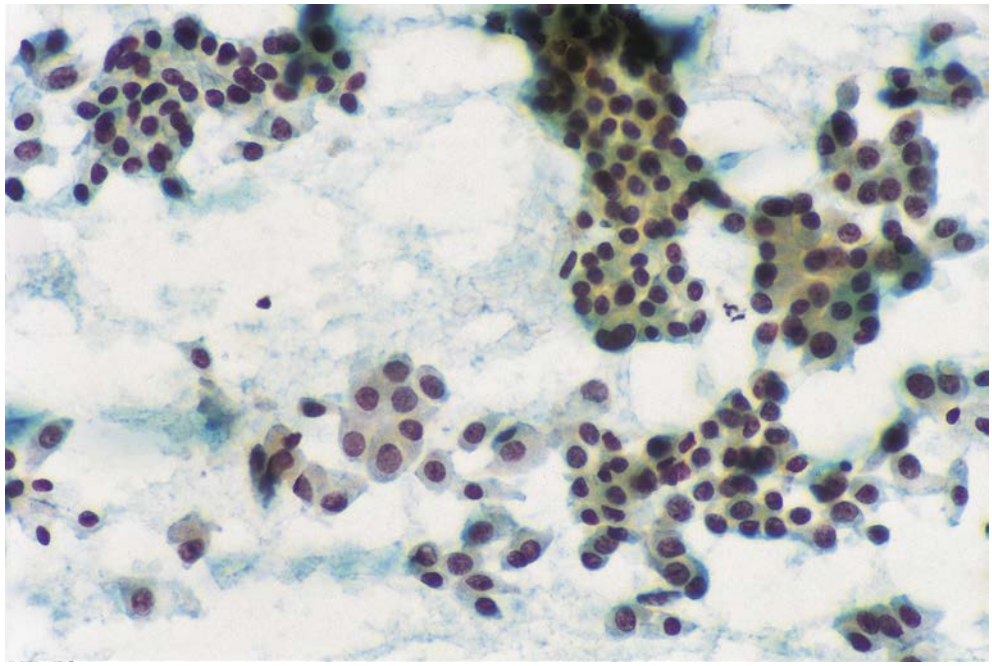

Fig. 11.39 Bile-duct epithelium. Clusters of bile-duct epithelial cells are distinguishable from the group of hepatocytes near the centre by their smaller size and round, non-descript nuclei. Microglandular structures are also focally present. (Papanicolaou.)

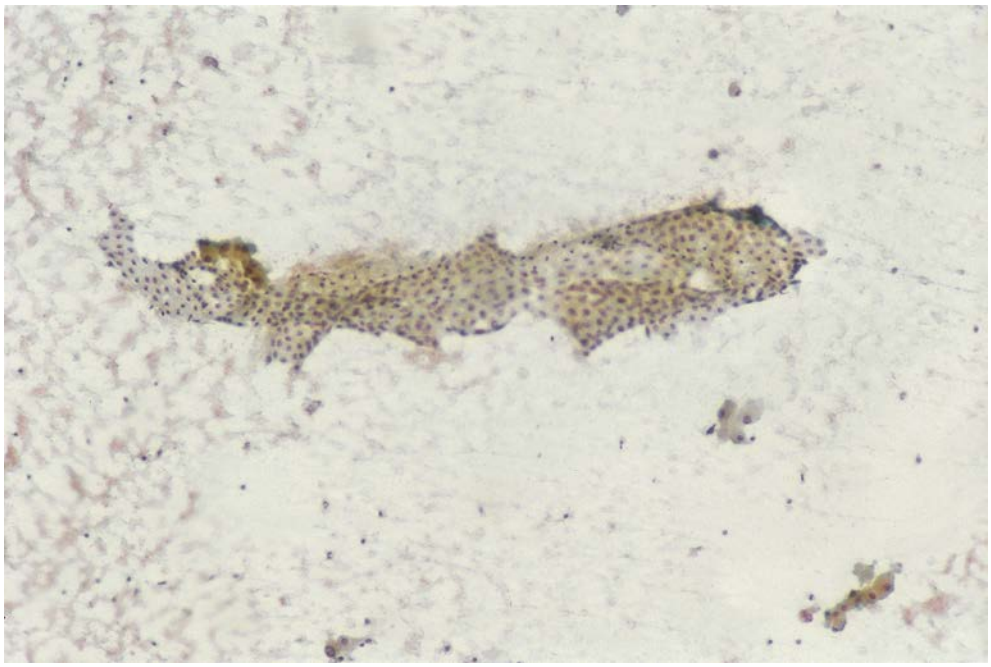

Fig. 11.40 Mesothelium. A sheet of mesothelial cells from the peritoneum shows a characteristic clear 'window' at right. (Papanicolaou.)

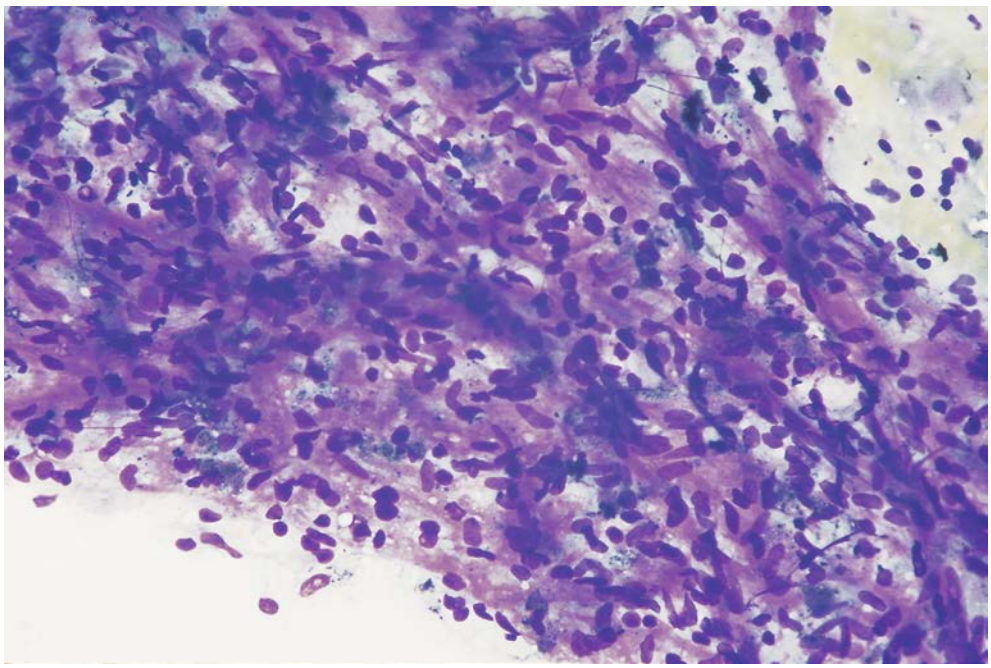

Fig. 11.41 Cirrhosis. The aspirate includes interweaving spindled fibroblasts, stroma and the round nuclei of lymphocytes. (Diff-Quick.)

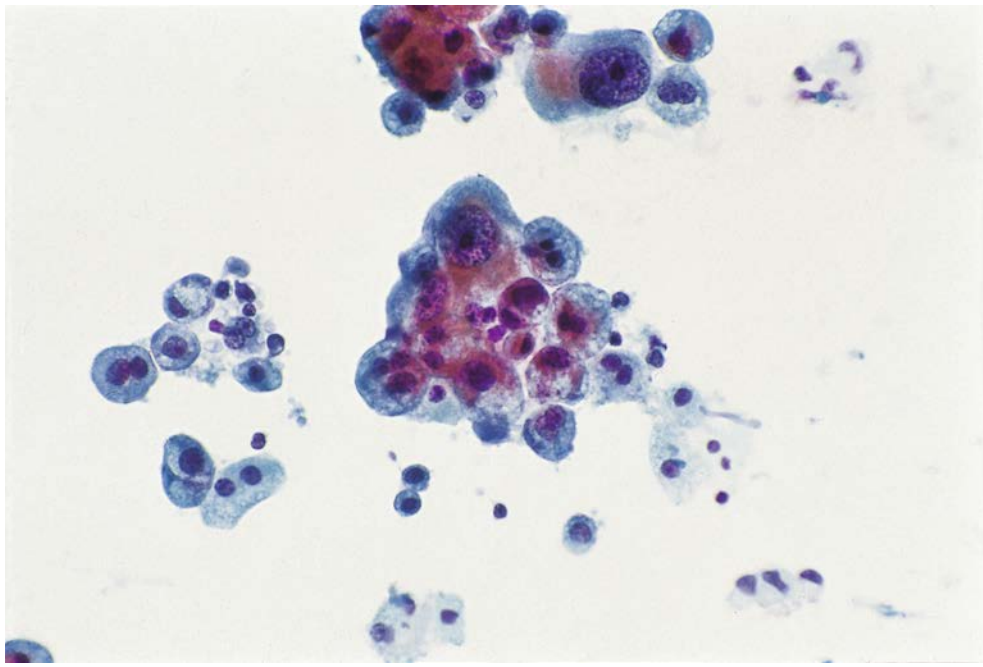

Fig. 11.42 Large-cell dysplasia. Two groups of hepatocytes (top and centre) contain large dysplastic cells intermixed with smaller reactive hepatocytes. The dysplastic cells have hyperchromatic nuclei, coarse chromatin and prominent nucleoli. Normal hepatocytes are seen at left and at bottom. (Papanicolaou.) (Illustration kindly provided by Dr Alastair Deery, London, UK.)

in cells that are smaller than normal hepatocytes (**Fig. 11.43**). The aspirated cell clusters in which dysplastic hepatocytes may be found are accompanied by normal or reactive hepatocytes with heterogeneous nuclear features and cell sizes, an important distinction from aspirates of HCC, which typically show a relatively monomorphous population of hepatocytes.[440]

Hepatocellular adenoma

The cytological diagnosis rendered from the FNAB smear of HCA is typically 'compatible with' this tumour, because the specimen usually shows single hepatocytes or clusters which resemble benign, normal liver cells.[438] Bile-duct epithelium and connective tissue should be absent, in contrast to FNH.

Focal nodular hyperplasia

Establishing the cytological diagnosis of FNH is based on finding one or more of the several cellular elements constituting this lesion (hepatocytes, fibrous tissue, bile-duct epithelium) within the smear. The presence of bile-duct epithelium in duct-like structures or clusters effectively rules out liver-cell adenoma and HCC.[441] The bile-duct epithelium may show nuclear variation and conspicuous nucleoli.[441] Fibrous tissue and bile-duct epithelium are not always present in the aspirate, but the bland appearance of the hepatocytes—with small, round nuclei lacking prominent nucleoli—implies a benign lesion. The hepatocytes are arranged in clusters with irregular or jagged edges without traversing or peripheral endothelium.

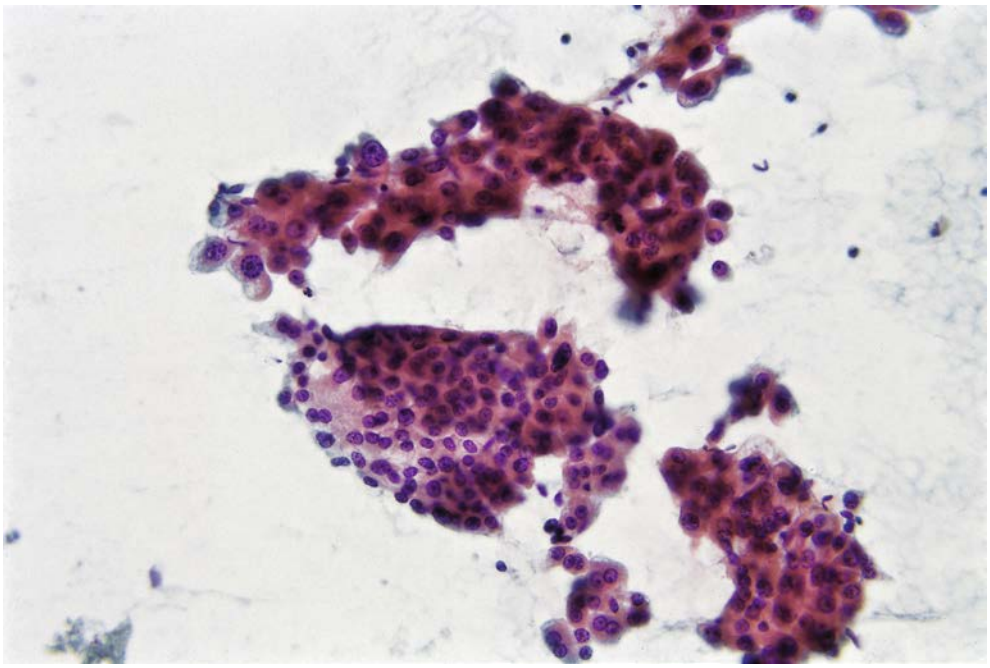

Fig. 11.43 Small-cell dysplasia. The cluster of small hepatocytes slightly below centre shows hyperchromatic atypical nuclei. (Papanicolaou.) (Illustration kindly provided by Dr Alastair Deery, London, UK.)

Haemangioma

Aspirates of haemangiomas are bloody, and numerous red blood cells are seen on the smear. Fragments of fibrous tissue[442] and/or single or clustered spindle-shaped endothelial cells may also be present (**Fig. 11.44**).

Hepatocellular carcinoma

The low-power microscopic appearance of smears from HCC provides several important diagnostic features, especially the rounded edges of tumour cells in clusters or trabeculae (in contrast to the ragged edges of normal or reactive hepatocyte clusters) and endothelial cells which traverse clusters of tumour cells as well as wrap around the periphery of clusters or trabeculae (**Figs. 11.45 and 11.46**). The paucireticulin pattern of HCC is helpful in FNABs where low-power examination of glass slides shows a finely granular smear (in contrast to the preserved cores or larger tissue fragments seen with benign liver diseases and masses[443]). Tumour cells are polygonal with central nuclei that may have either coarse or fine chromatin and prominent nucleoli or macronucleoli (**Fig. 11.47**). There is usually less variability in the features of HCC cells on smear than in normal or reactive hepatocytes.

Atypical naked nuclei[444] (exceptionally large and irregular nuclei without visible cytoplasm) are also an important diagnostic feature (**Fig. 11.48**). A stepwise logistic regression study showed that the three features which best differentiate HCC from normal or reactive liver are (1) increased nucleus-to-cytoplasm ratio; (2) a trabecular pattern of tumour cells enclosed by endothelium; and (3) atypical naked nuclei.[436,445] **Box 11.1** summarises some of the major FNAB cytological features of HCC. The presence of bile within or between tumour cells is indicative of their hepatocellular origin, but bile may be seen in only half the cases[446] and can also be present in smears from non-neoplastic liver.

Variants of HCC which may be present include acinar, clear-cell and fibrolamellar carcinoma. As with typical HCC, the presence of peripheral endothelial wrapping or traversal of endothelium across tumour cell clusters favours HCC. When **clear cells** are identified, there are also usually non-clear-cell HCC cells present which help distinguish the tumour

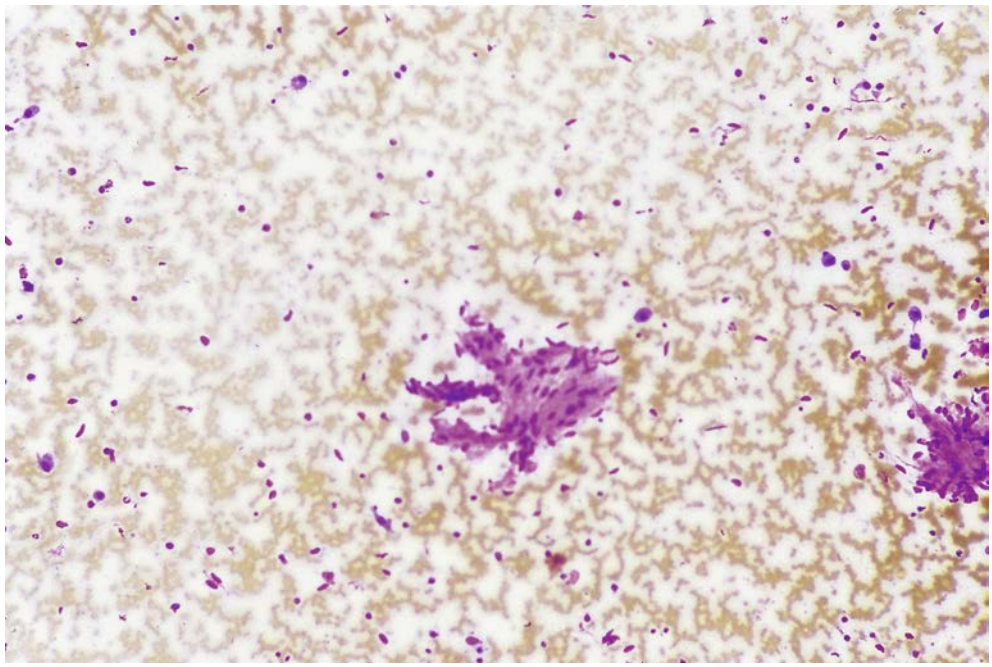

Fig. 11.44 Haemangioma. A focus of stromal cells is present with an extensive background of red blood cells. Scattered spindle-shaped endothelial or fibroblast nuclei are seen amid the erythrocytes. (Papanicolaou.)

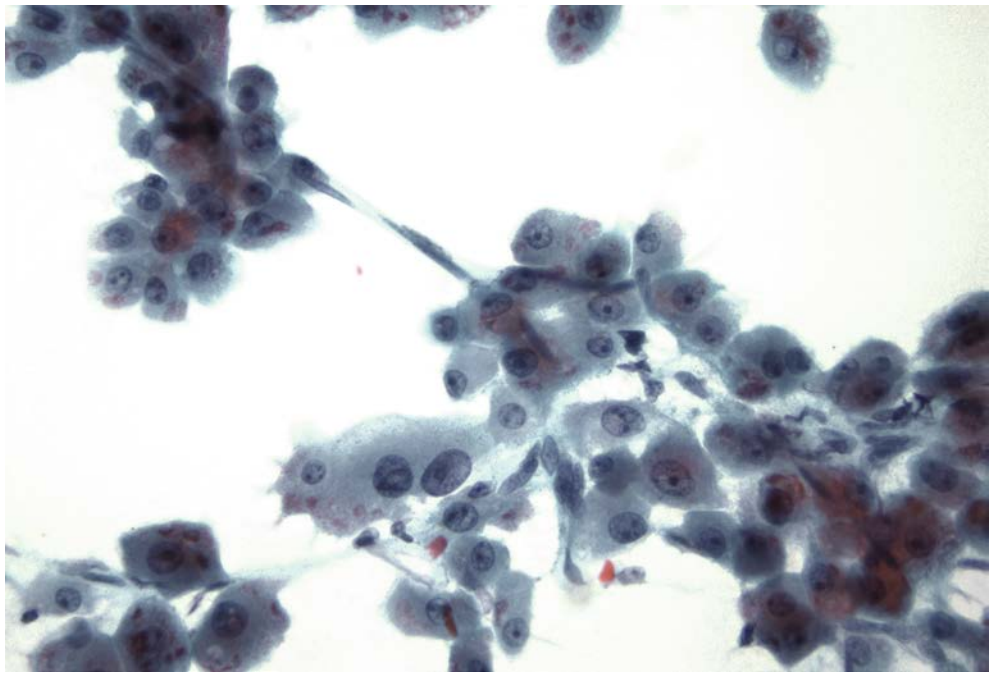

Fig. 11.45 Hepatocellular carcinoma. Malignant hepatocytes in clusters are traversed by slender strings of endothelium. (Papanicolaou.)

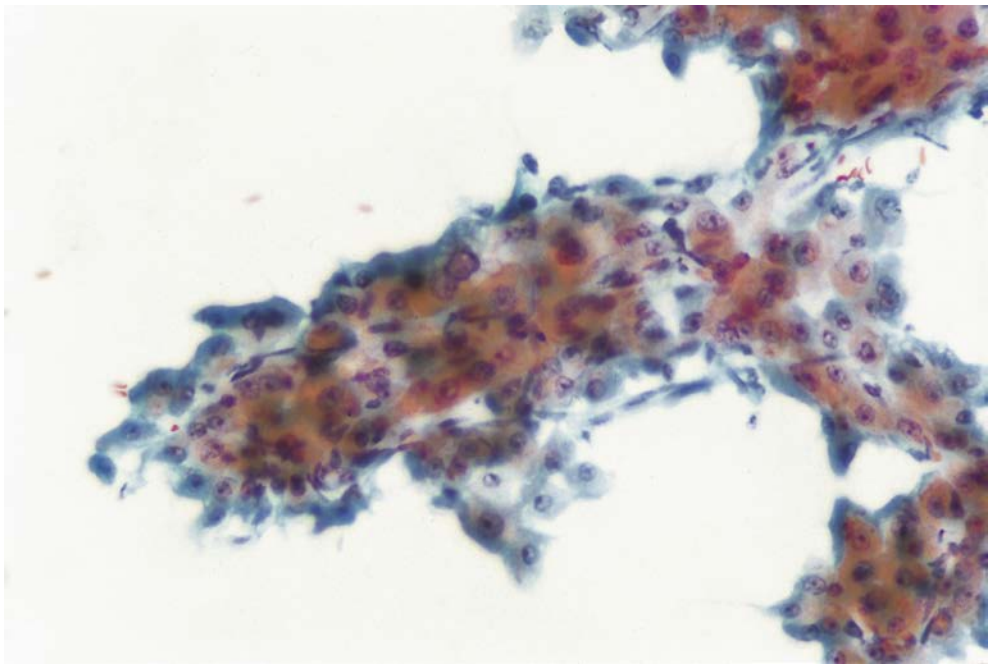

Fig. 11.46 Hepatocellular carcinoma. Flattened endothelium is seen peripherally at the edge of a trabecula of hepatocellular carcinoma. (Papanicolaou.)

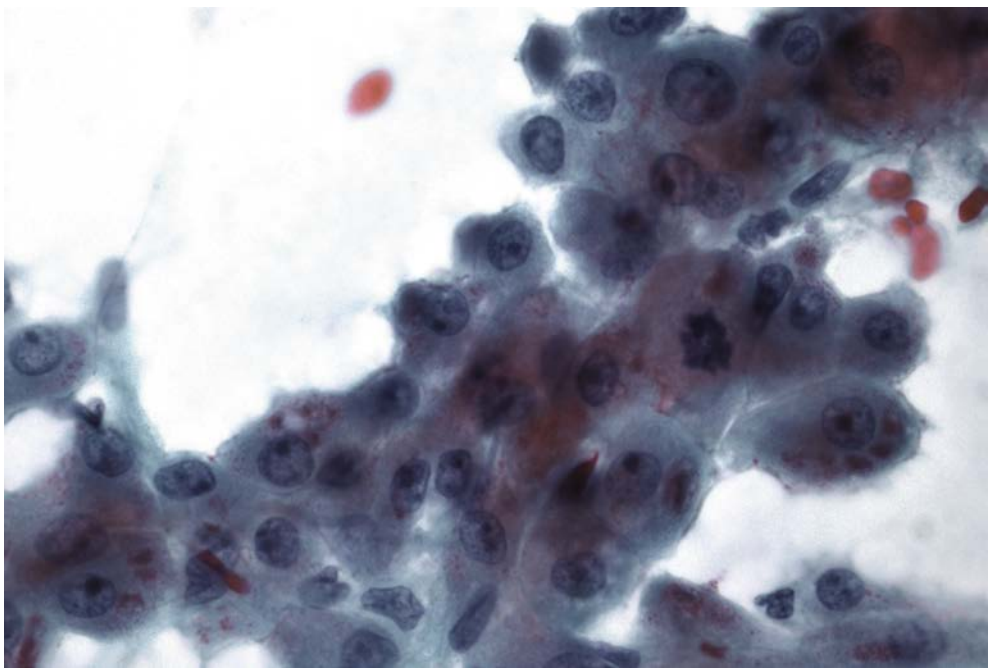

Fig. 11.47 Hepatocellular carcinoma. Malignant hepatocytes have fairly uniform nuclei which are centrally or peripherally located. Prominent nucleoli are seen throughout and a mitotic figure is present at right centre. (Papanicolaou.)

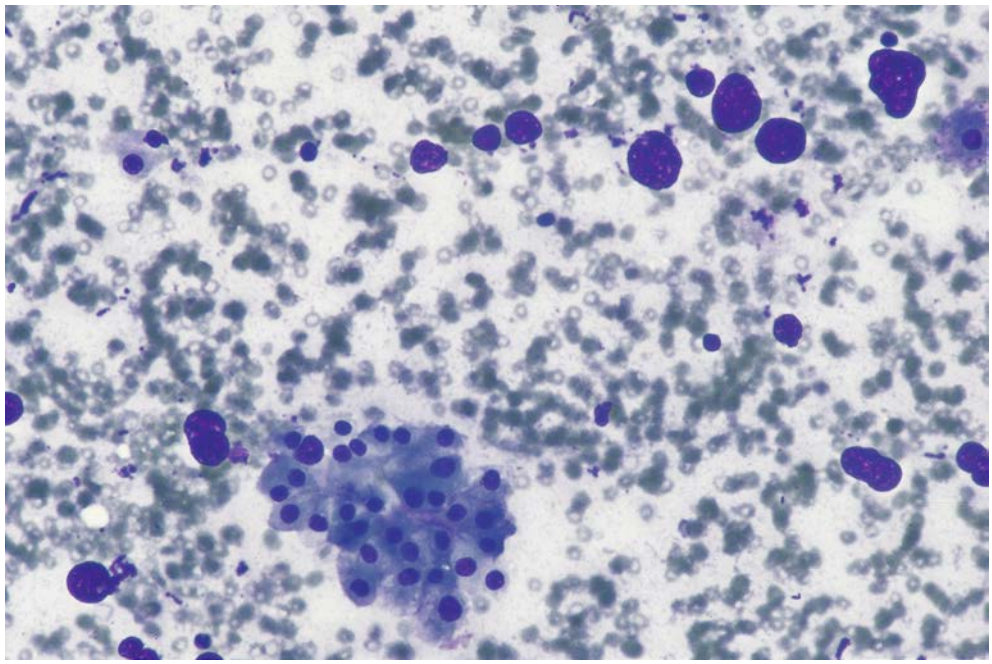

Fig. 11.48 Hepatocellular carcinoma. A cluster of malignant hepatocytes is present along with 'naked nuclei'. (Diff-Quick.)

Box 11.1 Major fine-needle aspiration biopsy features of hepatocellular carcinoma

Polygonal cells with central or paracentral nuclei
Relative homogeneity of tumour cells
High nucleus-to-cytoplasm ratio
Cell nests and trabeculae with smooth edges
Traversing and/or peripheral endothelium
Atypical naked nuclei

from renal, adrenal and ovarian neoplasms.[447] **Fibrolamellar carcinoma** can be diagnosed if the smear includes fibrous tissue or fibroblasts and the distinctive polygonal cells with granular, eosinophilic cytoplasm[448] (**Fig. 11.49**). The tumour cells of fibrolamellar carcinoma are often dispersed or discohesive, in comparison with the cell clusters and trabeculae of typical HCC.[449] Immunohistochemistry for CD68 may be helpful in selected cases.[288]

When metastatic carcinoma must be differentiated from HCC on FNAB, the cytopathologist should bear in mind that nearly all HCCs will have two or three of the following key diagnostic criteria[436]: polygonal cells with centrally placed nuclei, malignant cells traversed by sinusoidal capillaries, and bile.

Hepatoblastoma

The **epithelial type** of hepatoblastoma cytologically resembles HCC and on smear shows cohesive nests, sheets or trabeculae of malignant hepatocytes (**Fig. 11.50**). The tumour cells have hyperchromatic nuclei which may overlap and show prominent nucleoli. Fetal and embryonal subtypes are difficult to distinguish on FNAB alone.[450] There may be extramedullary haemopoiesis, formation of acini and naked tumour-cell nuclei.[450,451] The **mesenchymal type** of hepatoblastoma, or the mixed epithelial–mesenchymal variant, may be represented by spindle cells in the smear.

Cholangiocarcinoma

Aspirate smears from cholangiocarcinoma demonstrate three-dimensional clusters of atypical cells having a small amount of cytoplasm and nuclei with granular chromatin and one or more prominent nucleoli. Tumour cells may also be arranged in acini and cannot be readily distinguished on routine stains from metastatic pancreatic or other adenocarcinomas.[448]

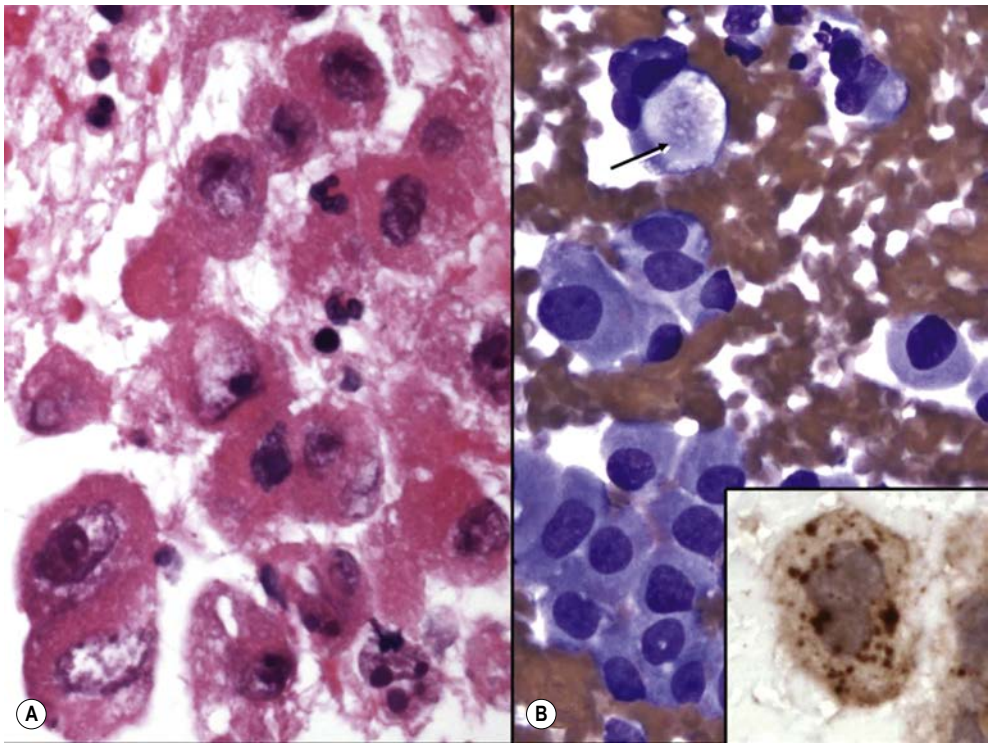

Fig. 11.49 Fibrolamellar carcinoma. A: A cell block obtained from a fine-needle biopsy aspirate shows neoplastic cells with ample eosinophilic and granular (oncocytic) cytoplasm and pleomorphic nuclei. (H&E stain.) **B**: The Diff-Quick stain of the aspirate shows plump tumour cells, one of which (at top) contains a 'pale body' (arrow). Inset: CD68 (KP1) immunostain of the cell block shows a tumour cell with positive stippled-granular staining of endosomes and lysosomes, as described in fibrolamellar carcinoma. (Specific immunoperoxidase.)

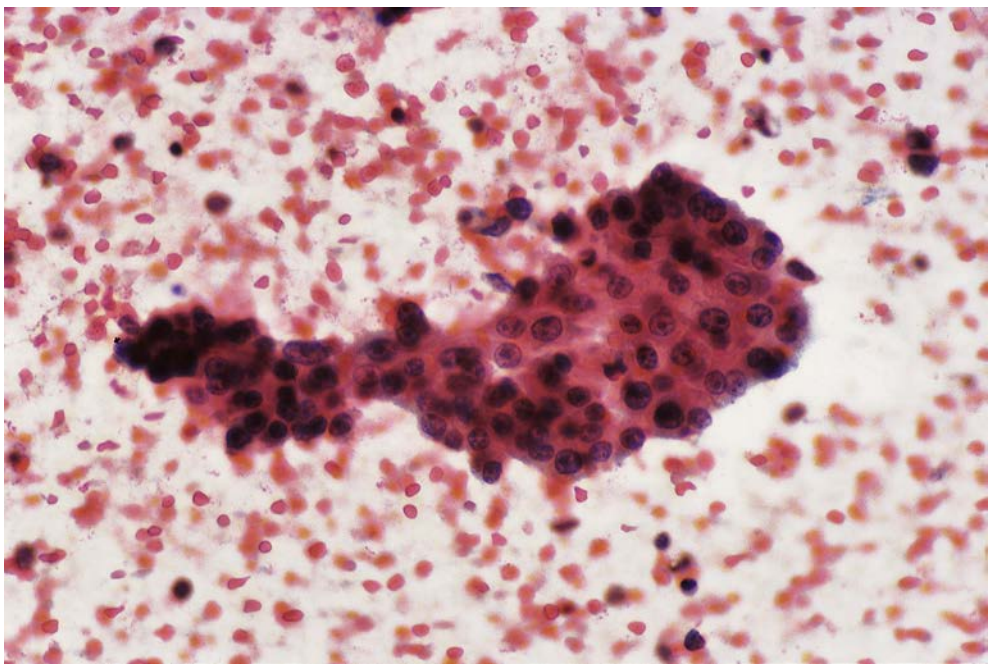

Fig. 11.50 Hepatoblastoma. The cell cluster is packed with malignant epithelium. Nuclear features resemble those seen in hepatocellular carcinoma. (Papanicolaou.)

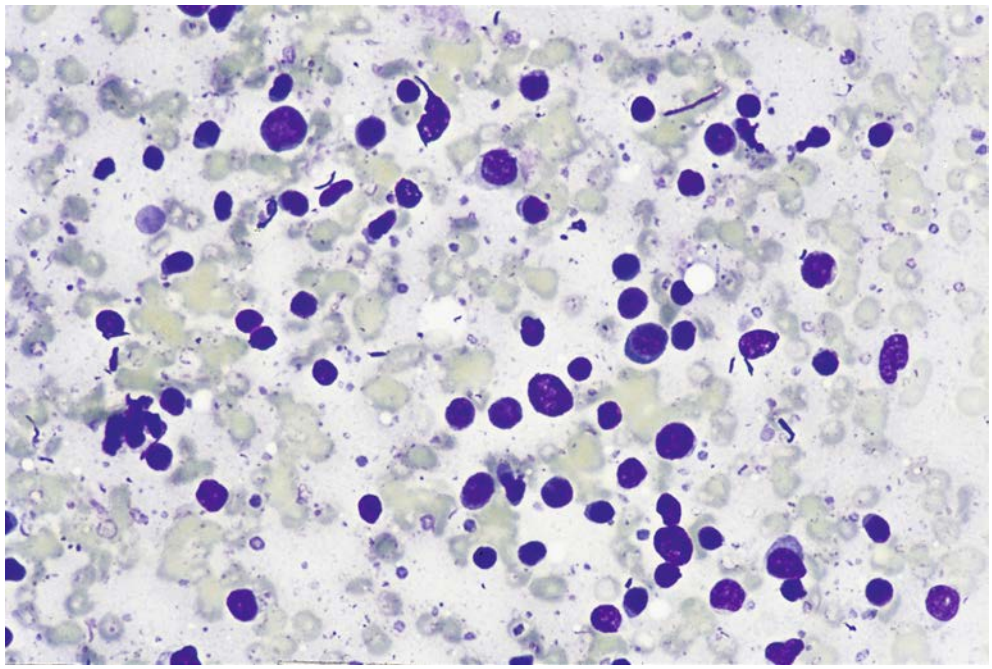

Fig. 11.51 Lymphoma. Dissociated malignant lymphoid cells are present with a background of smaller 'blue blobs' (lymphoglandular bodies). (Diff-Quick.)

Angiosarcoma

The smear of this tumour is typically bloody, and features a necrotic background with discohesive, pleomorphic spindle cells. Factor VIII and CD34 immunostains are diagnostically helpful.[452]

Lymphoma

Lymphoid proliferations in the liver, including lymphoma and post-transplant lymphoproliferative disease,[453] can be diagnosed on FNAB according to cytological criteria used for aspirates from extrahepatic sites. Smears show dispersed single, monomorphous lymphoid cells with 'blue blobs' of stripped cytoplasm (lymphoglandular bodies[454]) in the background (Fig. 11.51). Blue blobs may also be present in smears containing non-neoplastic lymphocytes.

Metastatic tumours

Adenocarcinoma from the colon and pancreas on aspirate smears presents as cell clusters with round or oval vesicular nuclei, prominent nucleoli and delicate cytoplasm. The presence of columnar cells with cigar-shaped, palisaded nuclei and apical cytoplasm or vacuoles (goblet cells) is characteristic of **colon carcinoma** (Fig. 11.52). The cytological features of **breast carcinoma** include the tendency to smear as discohesive cell groups or single cells with eccentric nuclei and cone-shaped cytoplasm. Relative uniformity of cell size and shape, prominent small nucleoli and the absence of marked atypia are seen in infiltrating duct carcinoma. Lobular carcinoma may show single-file lines of cells with nuclear moulding. **Neuroendocrine carcinomas** on smear feature organoid nests (carcinoid tumours) or loose groups or sheets (islet-cell carcinoma). The coarse 'salt-and-pepper' chromatin pattern is characteristic of these neoplasms, with islet cell carcinomas often showing more nuclear pleomorphism with prominent nucleoli than carcinoid tumours (Fig. 11.53). **Malignant melanoma** often metastasises to the liver and may be confused

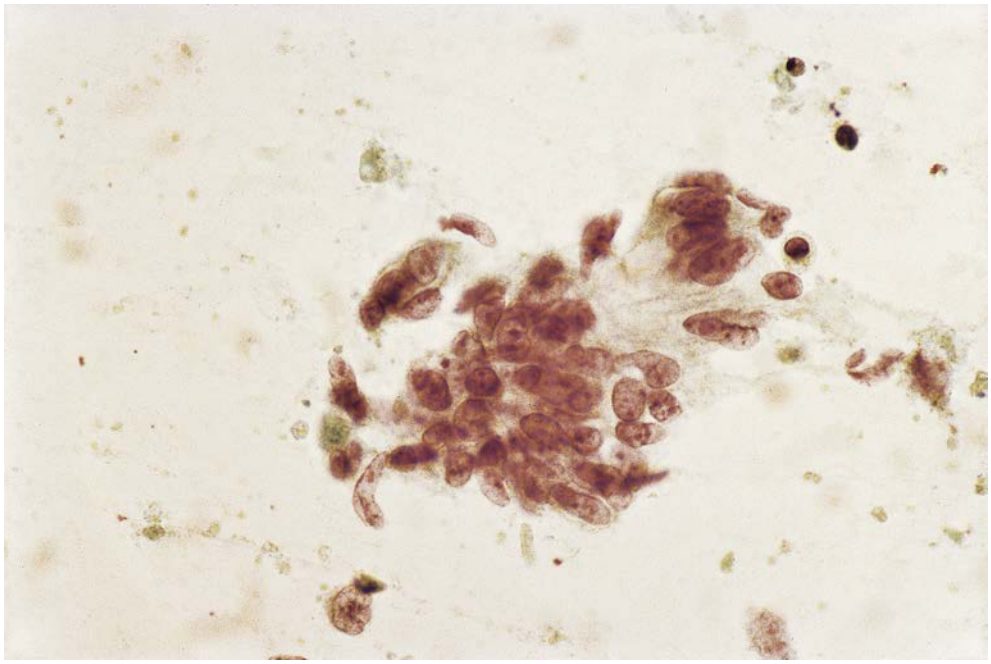

Fig. 11.52 Metastatic colonic adenocarcinoma. Several rows of columnar cells are present and the cluster at upper right contains a goblet cell with a large cytoplasmic vacuole. (Papanicolaou.)

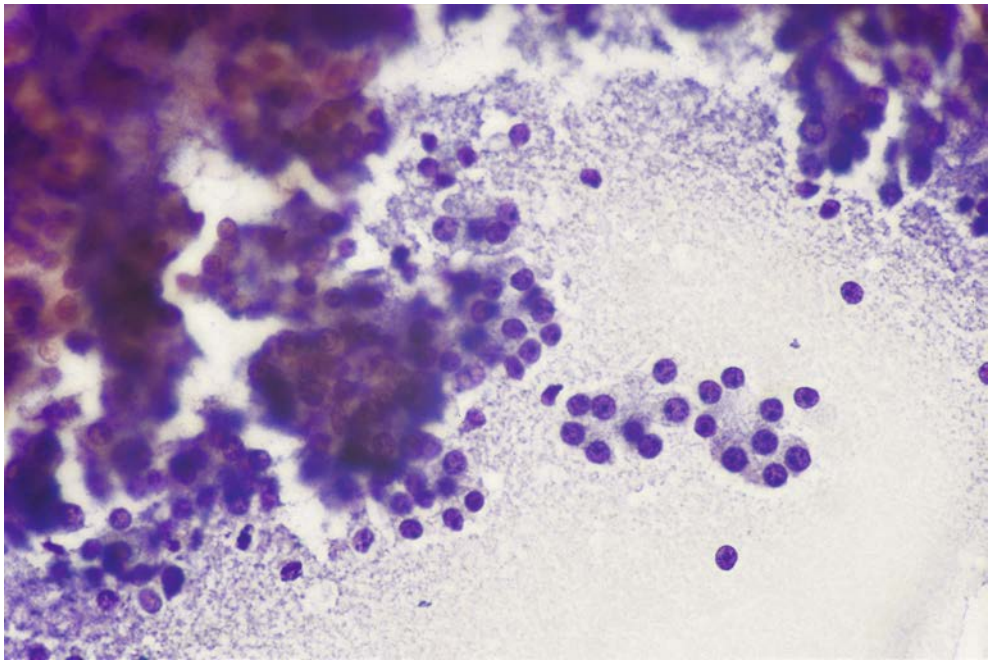

Fig. 11.53 Metastatic neuroendocrine carcinoma. Clusters of fairly homogeneous small cells with regular nuclei and substantial cytoplasm are seen. The patient had a pancreatic islet cell carcinoma. Thick trabecular structures are seen at left. (Diff-Quick.)

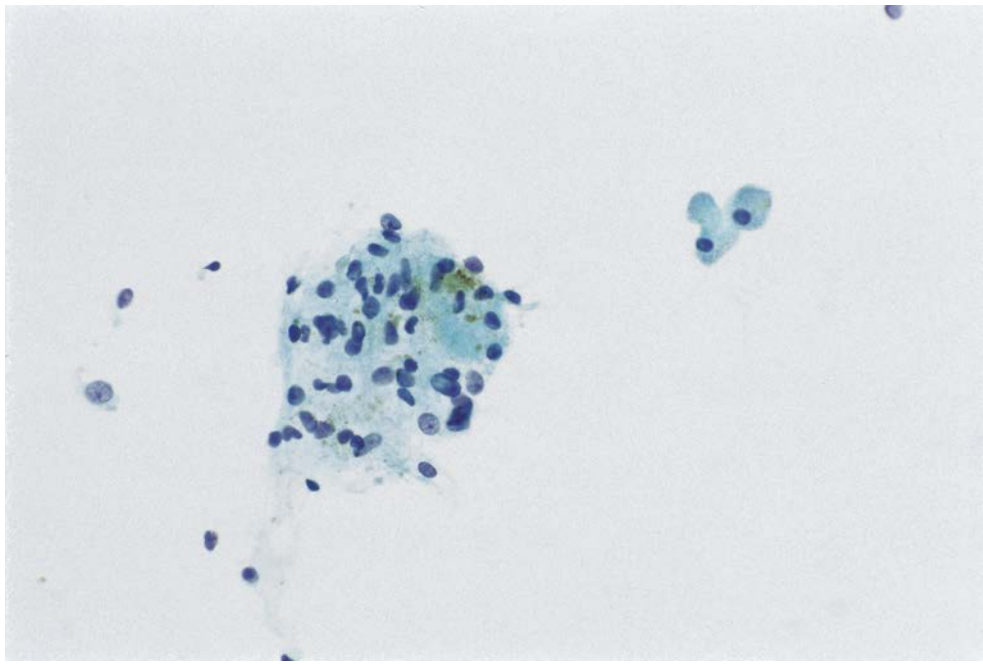

Fig. 11.54 Metastatic uveal melanoma. A cluster of melanoma cells with intracellular melanin pigment is present. Several normal hepatocytes are seen at upper right. (Papanicolaou.) (Illustration kindly provided by Dr Alastair Deery, London, UK.)

with HCC on FNAB because of features in common, including eosinophilic macronucleoli, nuclear pseudoinclusions, polygonal cell shape and cohesive cell groups. However, in contrast to HCC, aspirates of melanoma are more likely to show single, discohesive cells with eccentric nuclei and intracellular melanin pigment (Fig. 11.54). If cell grouping is present, it is unlikely to be accompanied by the traversing or peripheral endothelium seen in HCC. Immunostains for HMB-45 and S-100 should be undertaken if melanoma is a possible diagnosis, particularly if pigment is absent.

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CHAPTER

12 Vascular Disorders

The hepatic arteries

The effects of occlusion of hepatic artery branches are unpredictable because of the liver's double blood supply and variable collateral flow. Potential effects of thrombotic or other occlusion include infarction and ischaemic damage to the biliary tree leading to stricture formation, cholangitis or duct rupture.[1–3] The branches of the hepatic artery are sometimes involved in **polyarteritis nodosa** (**Fig. 12.1**),[2,4,5] the arteritis of **systemic lupus erythematosus**,[6] **Schönlein–Henoch purpura**[3] and **giant-cell arteritis**.[7] In the last, the liver may contain granulomas of classical[8] or fibrin-ring type.[9] The arterial lesions of these systemic diseases are not often seen in needle biopsies of the liver. Vasculitis affecting small intrahepatic vessels is sometimes a manifestation of infection or neoplasia.

In some older patients, especially those with systemic hypertension, small arteries and arterioles in portal tracts appear thickened and hyaline.[10] Patients with diabetes and hypertension may show an arteriolar microangiopathy, *hyaline arteriolosclerosis*, with increased wall thickness and deposition of periodic acid–Schiff (PAS)-positive material within the vessel wall.[11] **Amyloidosis** can give rise to thickening of arterial walls in the absence of sinusoidal deposits.

The arteriovenous malformations and telangiectases of **hereditary haemorrhagic telangiectasia** are sometimes found in the liver, with or without surrounding fibrosis.[12] The presentation is as portal hypertension (accompanied by hepatic encephalopathy and nodular regeneration[13,14]), biliary disease (sometimes resembling primary sclerosing cholangitis or Caroli's disease) or cardiac failure due to arteriovenous shunting.[15] Patients with liver involvement may have raised serum alkaline phosphatase levels without jaundice (anicteric cholestasis), attributed to abnormal blood supply to the biliary tree.[16a] Severely damaged medium-sized bile ducts are occasionally seen histologically.

Infarcts of the liver result from arteritis, aneurysms, thrombosis, embolism or surgical ligation. They may complicate pregnancy or liver transplantation.[16b] Infarction can also follow occlusion of portal-vein branches,[17] and may even be found in the absence of demonstrable vascular obstruction. The pathological features are as in other organs: there are well-defined zones of coagulative necrosis with congested and inflamed borders (**Fig. 12.2**). Portal tracts may survive within the infarcted areas. Coagulative necrosis of the centres of cirrhotic nodules following hypoperfusion is sometimes called nodular infarction.

Shock, heart failure and heatstroke

Severe hypoperfusion of the hepatic parenchyma leads to necrosis, usually in perivenular regions (acinar zone 3) but also, additionally or alternatively, in mid-zonal regions (zone 2).[18] Portal tracts and the periportal parenchyma typically remain normal. However, there are uncommon instances where interlobular bile ducts are injured due to hepatic hypoperfusion, especially in severely ill individuals with prolonged intensive care hospitalisations, and changes similar to sclerosing cholangitis may develop.[19,20] In contrast to the necrosis of acute hepatitis there is usually little or no inflammation, but in some patients neutrophils accumulate in limited numbers, particularly if the individual has received pressor support for 1 day or more.[21] Affected areas may be congested, and contain large, ceroid-laden macrophages. There may be cholestasis and evidence of regenerative hyperplasia in the surviving parenchyma. The reticulin network shows regular condensation in the necrotic areas. Similar changes are seen in patients with heatstroke (**Fig. 12.3**). There may be steatosis in the surviving parenchyma. Inflammation ranges from absent in mild cases[22] to severe when the damage is extensive.[23] Systemic candidiasis is a complication.

One of the most important causes of this type of necrosis is heart failure with consequent hypoperfusion of the liver. The term **ischaemic hepatitis** is commonly used for the viral hepatitis-like clinical picture which may ensue.[24] Congestive heart failure leads to sinusoidal dilatation (see the 'Venous congestion and outflow obstruction' section).

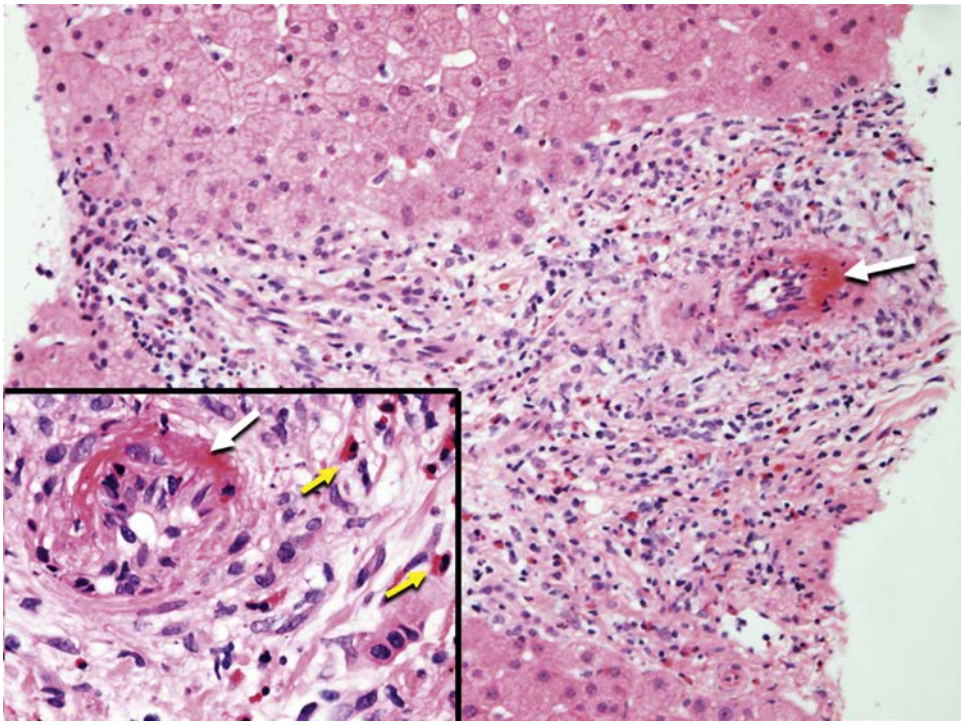

Fig. 12.1 Polyarteritis nodosa. The hepatic artery branch seen in cross-section in this markedly inflamed portal tract shows fibrinoid necrosis (white arrow) and scattered inflammatory cells. Inset: This deeper cross-section of the hepatic artery branch again demonstrates fibrinoid necrosis (white arrow) with scattered lymphocytes. The surrounding portal connective tissue is mildly oedematous and contains both lymphocytes and eosinophils (yellow arrows). (Needle biopsy, H&E.)

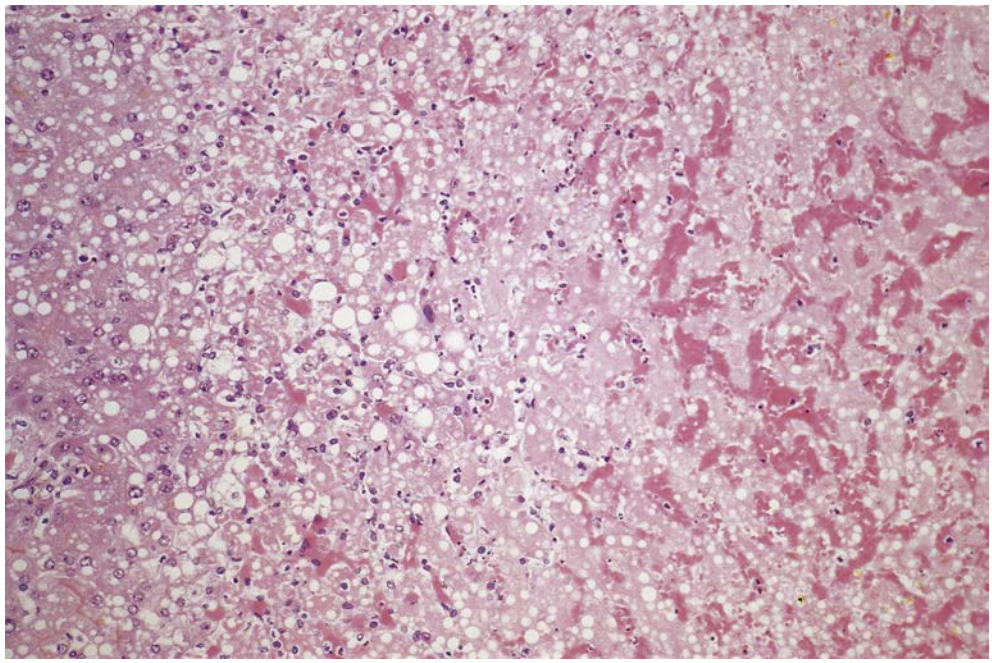

Fig. 12.2 Infarct. The dead parenchyma to the right is intensely congested. Surviving liver tissue (left) is fatty. (Postmortem liver, H&E.)

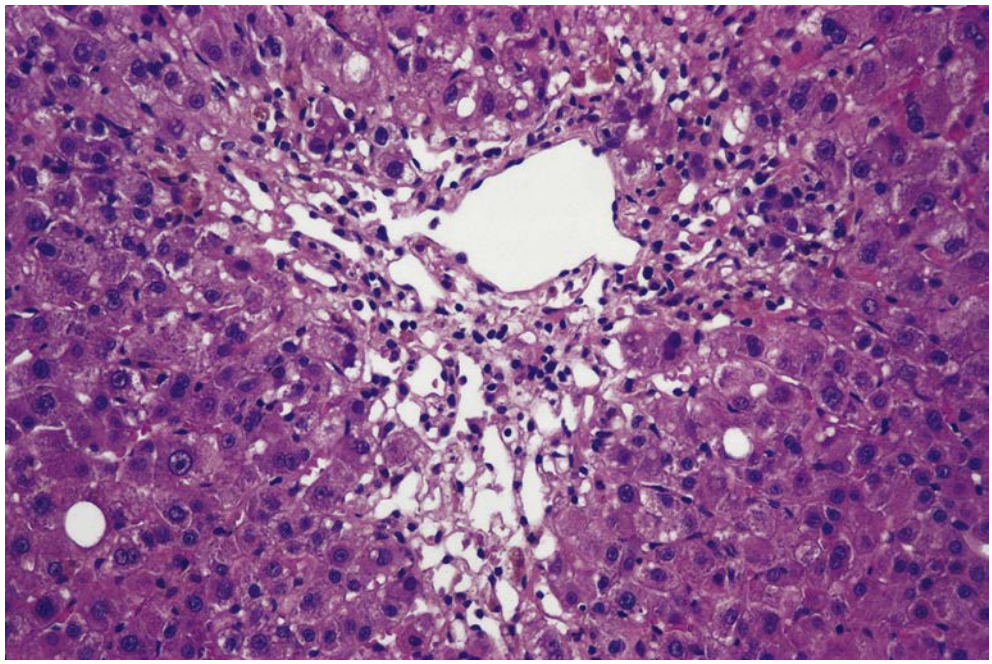

Fig. 12.3 Heatstroke. There has been confluent necrosis in acinar zone 3. (Needle biopsy, H&E.) (Section kindly provided by Professor Helmut Denk, Graz, Austria.)

The portal veins

Thrombosis of the main portal veins may result from infection (local or in the portal venous drainage area), cirrhosis,[25] liver transplantation, disorders of coagulation and

venous outflow obstruction.[26] Invasion by hepatocellular carcinoma is a common cause. In some patients no reason for the thrombosis can be discovered, but an underlying thrombophilic condition should always be excluded.[25] In the acute phase of pylephlebitis, septic thrombi may be seen in portal-vein branches in portal tracts (**Fig. 12.4**).

Possible results of portal-vein thrombosis include diffuse or focal parenchymal atrophy, increase in the number of apoptotic hepatocytes,[27] parenchymal nodularity (see the 'Nodular regenerative hyperplasia' section in **Ch. 11**) and a mild degree of portal fibrosis. Focal atrophy, also known as Zahn's infarction, is often found at the margins of tumour nodules. Occasionally, portal venous obstruction leads to true infarction of the hepatic parenchyma.[17] In many patients with thrombosis of the main portal veins the liver remains histologically normal.

Portal-vein branches are absent from portal tracts in the rare **Abernethy malformation** (congenital extrahepatic portosystemic shunts). In this condition the extrahepatic portal vein is either absent or severely atrophic, and portal blood is diverted to the inferior vena cava, rather than returning to the heart via the liver. Intrahepatic small portal tracts show absent veins, fibrous vein remnants, dilated lymphatics and arteriolar changes (**Fig. 12.5**).[28] Various nodular lesions including focal nodular hyperplasia, hepatocellular adenoma and hepatocellular carcinoma (with beta-catenin mutations) may develop.[29,30]

Portal hypertension

Portal hypertension is most often the result of cirrhosis. Other causes include schistosomiasis, alcohol-related liver disease, non-alcoholic steatohepatitis, congenital hepatic fibrosis, the tropical splenomegaly syndrome, hepatic venous outflow obstruction and portal venous thrombosis. The last probably contributes to portal hypertension in polycythaemia and other haematological diseases.[31] In lymphoproliferative and myeloproliferative disorders, the portal infiltration may be a further pathogenetic factor.[32] The anatomical subdivision of portal hypertension into prehepatic, intrahepatic and posthepatic forms should be considered in conjunction with specific structural alterations in classifying the individual case.[33]

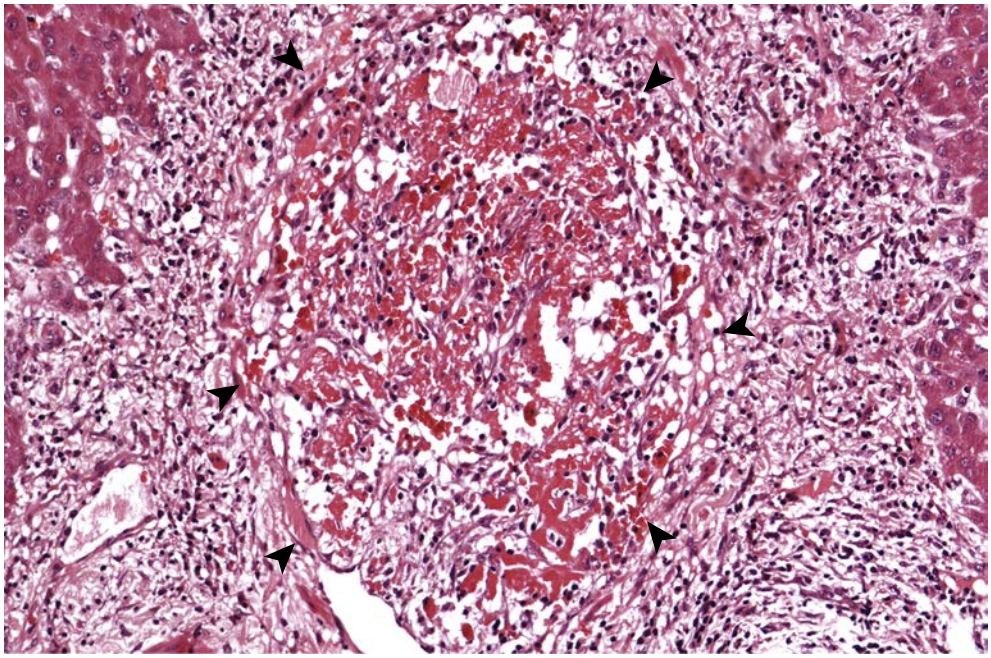

Fig. 12.4 Pylephlebitis. Thrombus with inflammatory cells, outlined by arrowheads, fills a portal-vein branch. The surrounding portal tract is also inflamed. (Wedge biopsy, H&E.)

There remains a somewhat ill-defined group of patients with portal hypertension not attributable to cirrhosis or to the other causes mentioned earlier (**non-cirrhotic portal hypertension**[34]). These cases represent an intrahepatic type of portal hypertension[35] which is a category separate from prehepatic causes such as thrombosis of the major portal vein and from posthepatic causes such as congenital webs of the inferior vena cava. Several different labels have been used to describe aspects of this group **(hepatoportal sclerosis, non-cirrhotic portal fibrosis, idiopathic portal hypertension)**. The term **obliterative portal venopathy** has also been used[36] and indicates that there may be demonstrable thrombosis or narrowing of portal-vein branches, but this is not always the case, and it is not clear whether the portal venous narrowing or occlusion is primary or secondary. Non-cirrhotic portal hypertension is most prevalent in India and Japan, but is also described in Western countries.[35] Certain cases have been attributed to a toxin or toxins such as arsenic,[35,37] vinyl chloride,[38,39] azathioprine,[31] cytotoxic drugs[36,40] and didanosine therapy in human immunodeficiency virus (HIV) disease.[36] Thrombophilic/pro-coagulant states need to be excluded as possible causes. In many patients no cause is found. Variceal bleeding and portal-vein thrombosis are important long-term complications.[41]

Needle liver biopsies from patients with non-cirrhotic portal hypertension are often normal or show only non-specific changes. Abnormalities are more likely to be seen in operative wedge biopsies. Portal-vein branches are sometimes thickened and narrowed, unusually inconspicuous or replaced by multiple small, thin-walled channels. Their overall area is reduced, while portal tract lymphatics increase in number.[42] Dilated venules appear to herniate into the adjacent parenchyma[43,44] (**Fig. 12.6**). There may be portal fibrosis and enlargement, with or without inflammatory-cell infiltration (**Fig. 12.7**). Slender fibrous septa extending from the portal tracts give an appearance indistinguishable from incomplete septal cirrhosis.[45] These septa sometimes connect with bridge-like zones of necrosis.[43] There may be randomly distributed thin-walled vessels in the lobules (megasinusoids), and sclerosis or dilatation of efferent veins.[43]

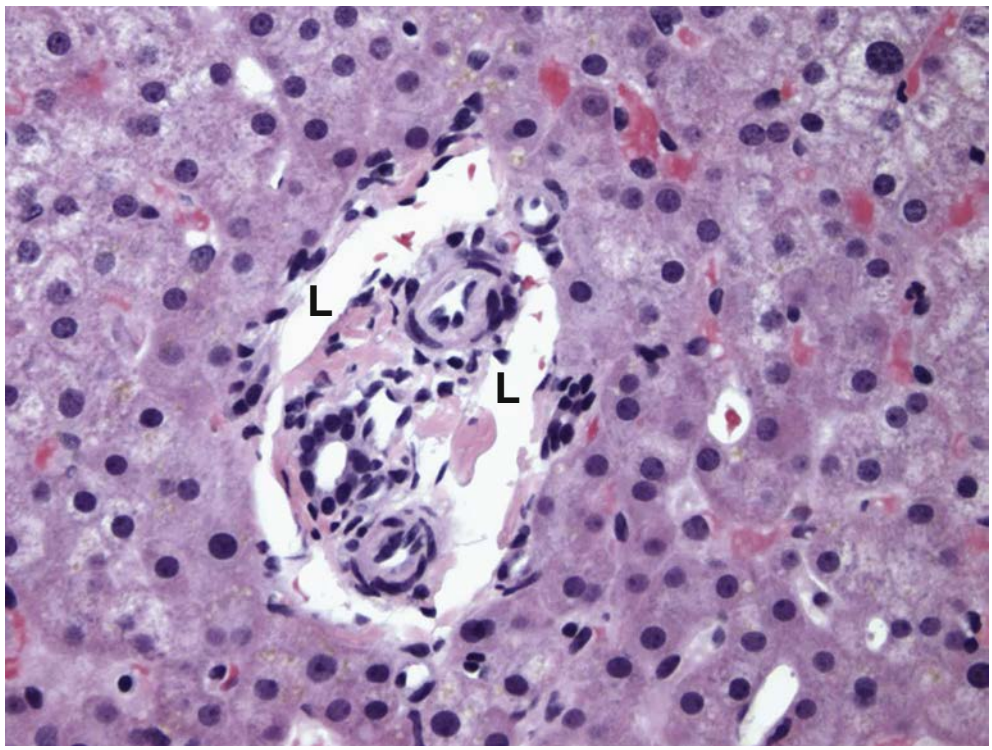

Fig. 12.5 Portal-vein absence in Abernethy malformation. The portal tract shows an interlobular bile duct and two cross-sectional cuts of the hepatic arteriole, but no typical large-calibre portal vein is present. The prominent spaces adjacent to the portal tract are dilated lymphatics (L), which showed endothelial positivity with D2-40 immunostain. (Explant liver, H&E.)

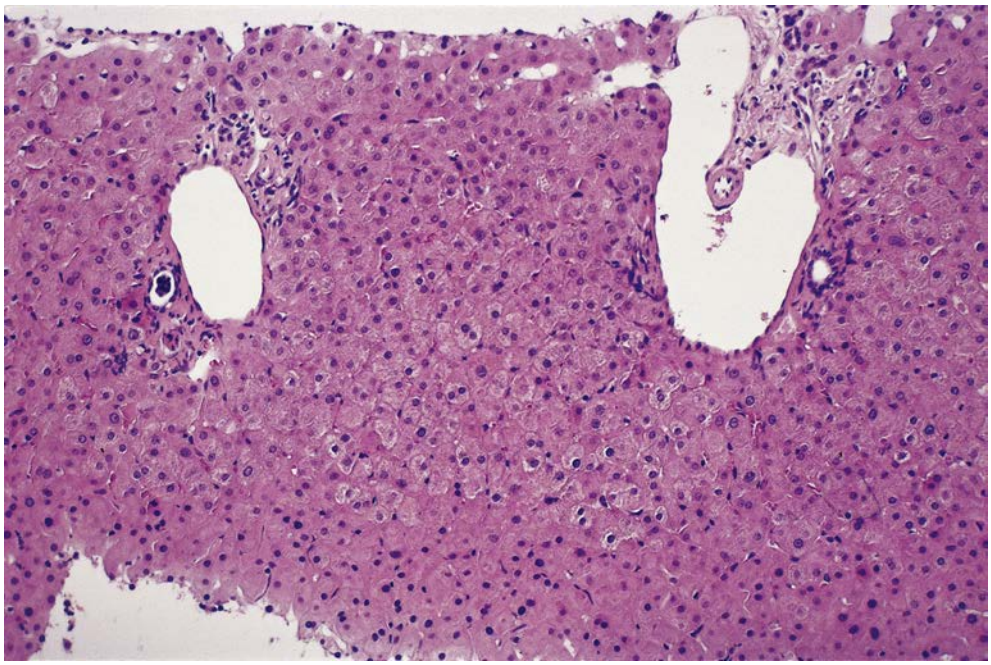

Fig. 12.6 Non-cirrhotic portal hypertension. The portal-vein branches in the two portal tracts are widely dilated and appear to have herniated into the parenchyma. (Needle biopsy, H&E.) (Section kindly provided by Professor Helmut Denk, Graz, Austria.)

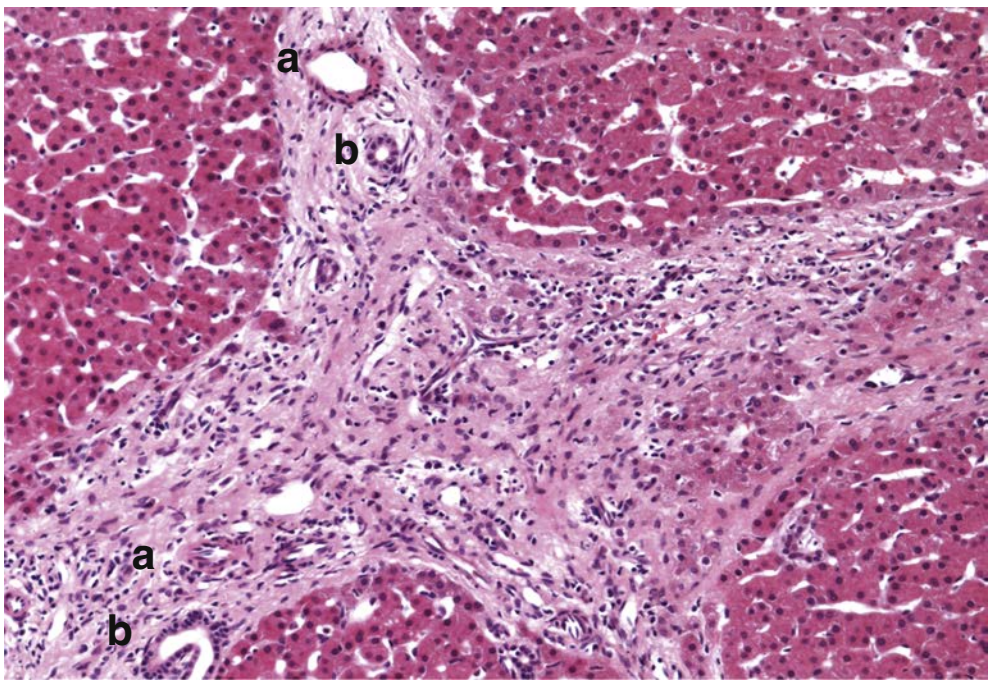

Fig. 12.7 Non-cirrhotic portal hypertension. An enlarged, sclerotic portal tract contains arteries (a) and bile ducts (b), but portal-vein branches are inconspicuous. (Wedge biopsy, H&E.)

Diffuse or localised nodular hyperplasia of the parenchyma is commonly seen in these patients. There is thus overlap between hepatoportal sclerosis, nodular regenerative hyperplasia, incomplete septal cirrhosis[45,46] and, rarely, partial nodular transformation.[47] Nodular regenerative hyperplasia, however, is also found in the absence of clinically evident portal hypertension.

In patients exposed to vinyl chloride monomer and other carcinogens there may be, in addition to the aforementioned features, perisinusoidal fibrosis and an increase in the number and size of sinusoidal cells.[38,39] Perisinusoidal fibrosis may also contribute to the portal hypertension which develops in some patients after renal transplantation.[48] Prolonged drug therapy has been suggested as a possible mechanism.

The hepatic sinusoids

The sinusoids may show a spectrum of pathological changes, from dilatation and congestion to lesions affecting the subendothelial spaces of Disse.[49] The width of the sinusoids in liver biopsy specimens is very variable. It is influenced not only by the state of the patient's circulation at the time of biopsy but also by fixation and tissue processing. Slight variations in width are therefore of doubtful significance.

The amount of connective tissue in sinusoidal walls should also be assessed critically, because its appearance varies with section thickness. A definite increase in fibres is characteristic of chronic venous outflow obstruction and of steatohepatitis. In the former the pattern of fibrosis is usually linear (peri- or parasinusoidal fibrosis), while in steatohepatitis the fibrosis surrounds hepatocytes (pericellular fibrosis). Increased sinusoidal type IV collagen in diabetic hepatosclerosis[50] is also in the differential diagnosis (**Ch. 7**). Other causes and associations, some of them already mentioned earlier, include congenital syphilis, vinyl chloride toxicity, heroin addiction,[51] hypervitaminosis A,[52] diabetes,[53] renal transplantation, myeloid metaplasia[54] and thrombocytopenic purpura.[55] Similar perivenular, perisinusoidal fibrosis may also be sequelae following episodes of central perivenulitis associated with acute cellular rejection after liver transplantation (**Ch. 16**) and sometimes after centrilobular necroinflammation seen in the histological variant form of autoimmune hepatitis (**Ch. 9**). Endothelial cells lining the hepatic sinusoids sometimes contain iron-rich granules of uncertain significance, especially in viral hepatitis[56] and alcoholic liver disease.

Definite and regular **dilatation** of the sinusoidal network is associated with several conditions, the most important being venous outflow obstruction (discussed later). It has been reported in patients with tumours or granulomas, even when these did not involve the liver,[59] in Crohn's disease,[60] in patients with anticardiolipin antibodies and features of the antiphospholipid syndrome,[61] haemophagocytic syndrome[62] and in long-term heroin users.[63] Sinusoidal dilatation and congestion in the absence of venous outflow obstruction may also be seen with portal-vein thrombosis and congenital absence, rheumatoid arthritis, Still's disease, and in wedge biopsies taken during abdominal surgery.[64] Dilatation of periportal and mid-zonal sinusoids has been described in a small number of patients taking oral contraceptives[65,66] (**Fig. 12.8**). In some patients with renal cell carcinoma there is focal dilatation of mid-zonal sinusoids.[67]

Peliosis hepatis

The borderline between regular diffuse dilatation and the focal dilatation known as peliosis hepatis is not always sharp.[67,68] In peliosis, blood-filled cysts are found in the parenchyma (**Fig. 12.9**), ranging in size from less than 1 mm to several millimetres in diameter. The endothelial lining is usually incomplete.[69] Peliosis is found in association with many different conditions and circumstances, including wasting diseases, asphyxia,[70] neoplasia,[71] liver and renal transplantation,[72,73] drug therapy[74,75] and bacterial infection.[76] The lesion is often discovered incidentally, but rupture leading to haemoperitoneum has been reported.[76,77] Bacillary peliosis hepatis (**Ch. 15**) is a different lesion, and is attributed to the bacteria which cause cutaneous bacillary angiomatosis in patients with AIDS. Their presence in silver preparations distinguishes the condition from simple peliosis.

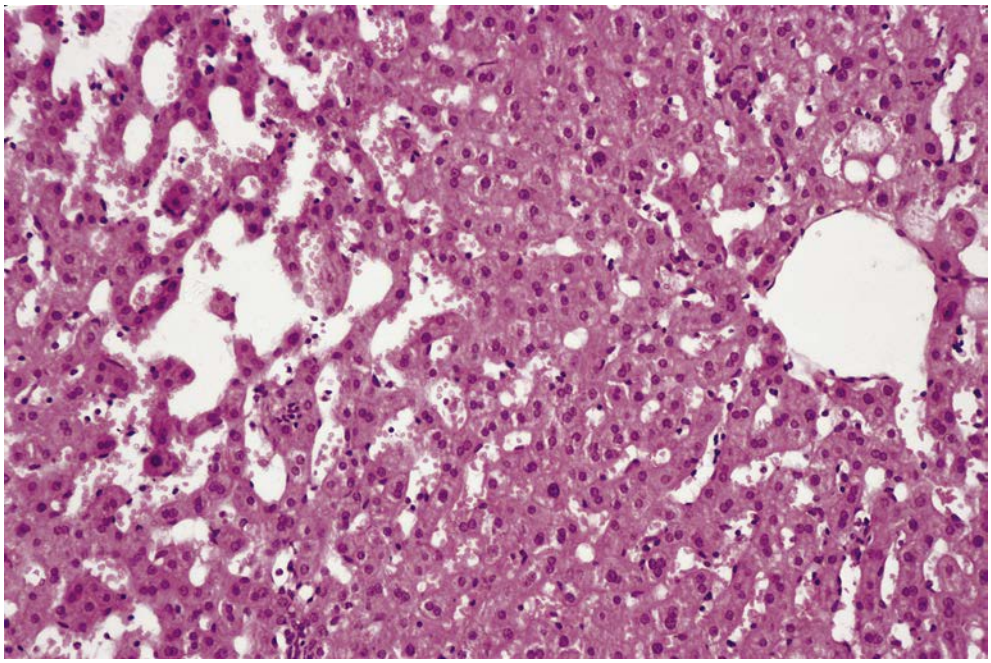

Fig. 12.8 Sinusoidal dilatation. Dilated periportal and midzonal sinusoids are seen to the left, and a terminal hepatic venule to the right. The dilatation was attributed to an oral contraceptive steroid. (Needle biopsy, H&E.)
(Section kindly provided by Professor Hemming Poulsen, Copenhagen, Denmark.)

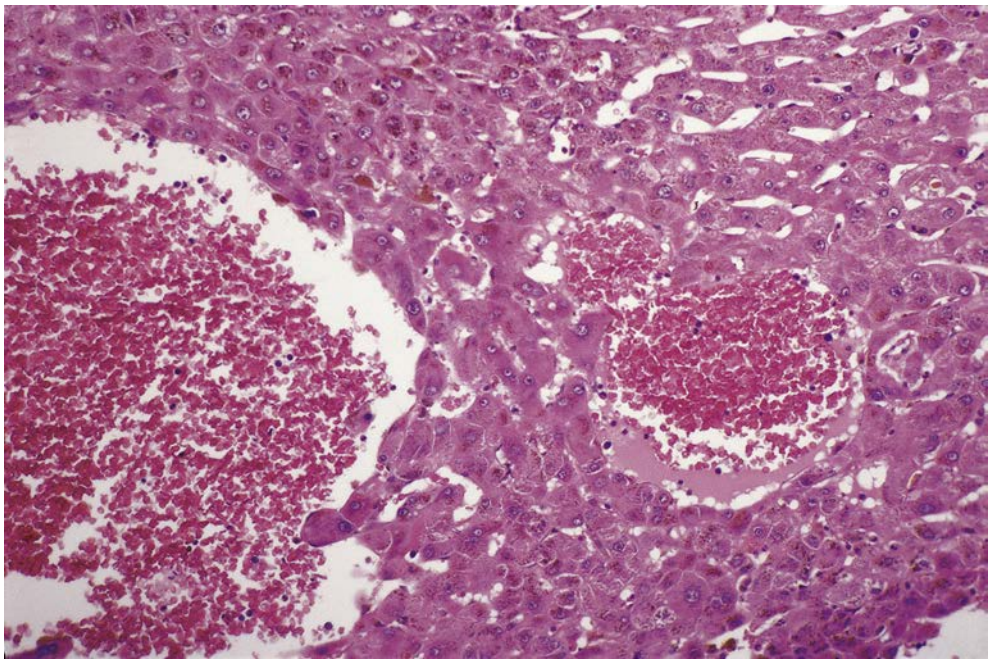

Fig. 12.9 Peliosis. There are blood-filled spaces within the parenchyma. (Needle biopsy, H&E.)

Disseminated intravascular coagulation

This commonly involves the liver.[78] Sinusoids and small portal vessels contain fibrin thrombi (**Fig. 12.10**), but the fibrin is often difficult to identify with certainty in conventional sections. Similar changes are seen in eclampsia, in association with periportal necrosis and acute inflammation. In congestive cardiac failure thrombi may form in the sinusoids.[79]

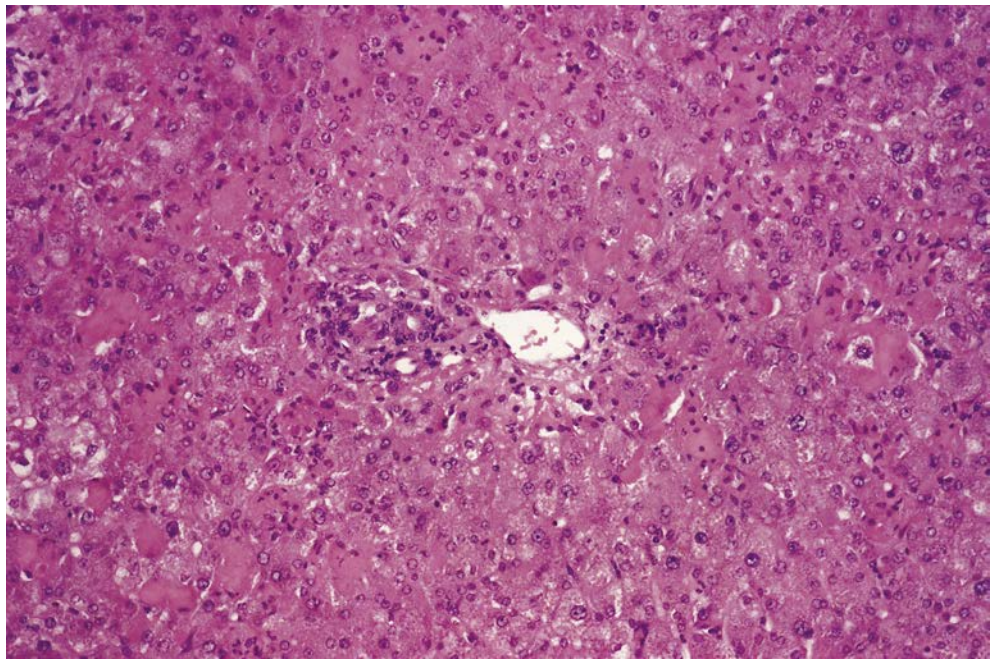

Fig. 12.10 Disseminated intravascular coagulation. Periportal sinusoids are filled with fibrin and neutrophils. (Needle biopsy, H&E.)

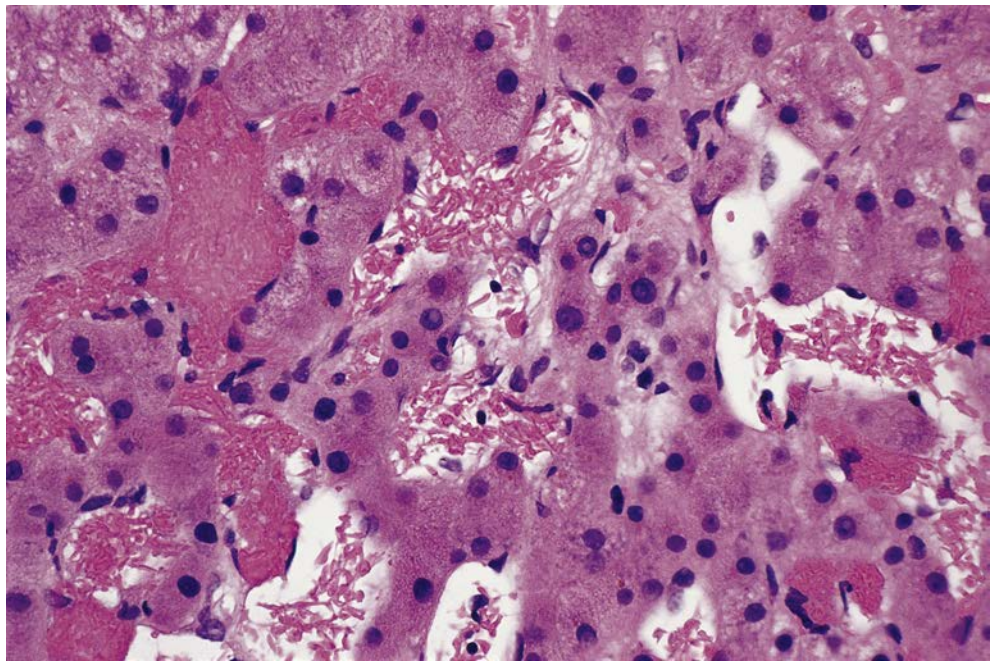

Fig. 12.11 Sickle-cell disease. Clumped and sickled erythrocytes are seen in distended sinusoids. (Needle biopsy, H&E.)

Sickle-cell disease

In most patients with sickle-cell disease, clumps of sickled erythrocytes are found in dilated sinusoids[80] (Fig. 12.11). Lesions of peliosis may develop, and there is often some degree of perisinusoidal fibrosis. There is erythrophagocytosis, and hypertrophied Kupffer cells and hepatocytes contain iron. Hepatocytes may show atrophy and ischaemic necrosis[81] as well as evidence of

regeneration. The degree of sickling seen does not correlate with biochemical or clinical evidence of liver damage, and some hepatic manifestations in patients with sickle-cell disease are thought to be the result of complications such as transfusion-related hepatitis,[82] siderosis, cholelithiasis and venous outflow obstruction. Cirrhosis occasionally develops, possibly as a consequence of viral hepatitis. Liver dysfunction in sickle-cell disease is sometimes due to autoimmune hepatitis and such cases show portal lymphoplasmacytic inflammation with interface hepatitis.[83]

Venous congestion and outflow obstruction

Interference with the venous outflow of the liver results from a multitude of causes, ranging from congestive cardiac failure to occlusion of the smallest tributaries of the hepatic veins within the liver. Space-occupying lesions such as tumours may cause localised obstruction affecting only parts of the liver. The term **Budd–Chiari syndrome** is often used to describe the clinical findings when the inferior vena cava or main hepatic veins are obstructed, and is sometimes extended to obstruction at the level of the heart.[84] Myeloproliferative neoplasms may cause thrombosis of hepatic veins, and these cases may harbour JAK2 (Janus kinase 2) gene mutations[85] or, infrequently, mutations in the gene for *calreticulin* (CALR).[85,86] Further classification should be based on the nature and location of the obstruction.[87,88] The pathologist faced with a severely congested liver biopsy is often unsure about the level and nature of the block. Use of the term **venous outflow obstruction** is then appropriate. Chronic venous outflow leads to fibrosis surrounding efferent veins and within nearby perisinusoidal spaces, eventually with variable degrees of bridging fibrosis (central-to-central or central-to-portal) and hepatocellular regenerative hyperplasia. The degree of fibrosis in chronic congestive hepatopathy should be stated in the biopsy report because it is an important parameter to correlate with cardiopulmonary and hepatic vascular pressures and with the possible need for double organ (i.e. heart–liver) transplantation (discussed further later)[89,90] Centrilobular regions may also demonstrate unusual changes, including ingrowth of microvessels,[91] aberrant immunohistochemical positivity of perivenular hepatocytes for biliary-type keratins[91,92] (cytokeratin 7) and altered glutamine synthetase immunostain results (either loss of usual expression in centrilobular hepatocytes or relocalisation of positivity to periportal/periseptal regions).[93] Hepatocellular carcinoma is an uncommon late outcome of chronic venous outflow obstruction in Budd–Chiari syndrome[94] and in young adults with single ventricle-type congenital heart diseases who have undergone Fontan procedures (discussed later).

Cardiac failure and congestive hepatopathy

The relative status of left versus right heart function influences changes seen in the liver microscopically. As discussed earlier, acute left ventricular dysfunction (as well as clinical shock of various aetiologies) results in hepatic hypoperfusion and hypoxia with coagulative necrosis of centrilobular hepatocytes (**Fig. 12.12**). Chronic cardiac disease with biventricular failure and other disorders associated with increased right heart volume/pressure load result in hepatic changes referred to clinically as *congestive hepatopathy*. The spectrum of histologic changes in congestive hepatopathy is fairly characteristic (**Box 12.1**). The terminal hepatic venules and adjacent sinusoids show variable combinations of dilatation and congestion.[95] The degree of dilatation or congestion can vary from lobule to lobule in a given tissue section. The congestion may be accompanied by hepatocellular necrosis if there is also a significant element of hypoperfusion (**Fig. 12.12**), as in the combination of right- and left-sided heart failure. Sinusoidal and venous thrombosis may also contribute to hepatocellular damage.[79] Blood may infiltrate the liver-cell plates.[96] Canalicular cholestasis is sometimes seen, and must be distinguished from the commonly found ceroid pigment in Kupffer cells. As indicated in the preceding section, centrilobular hepatocytes may show aberrant immunohistochemical positivity for the biliary-type cytokeratin 7.[91,92] Inflammation is typically mild or absent, and portal tracts usually remain normal. However, in some cases portal tract inflammation and a ductular reaction develop and may be mistaken for biliary

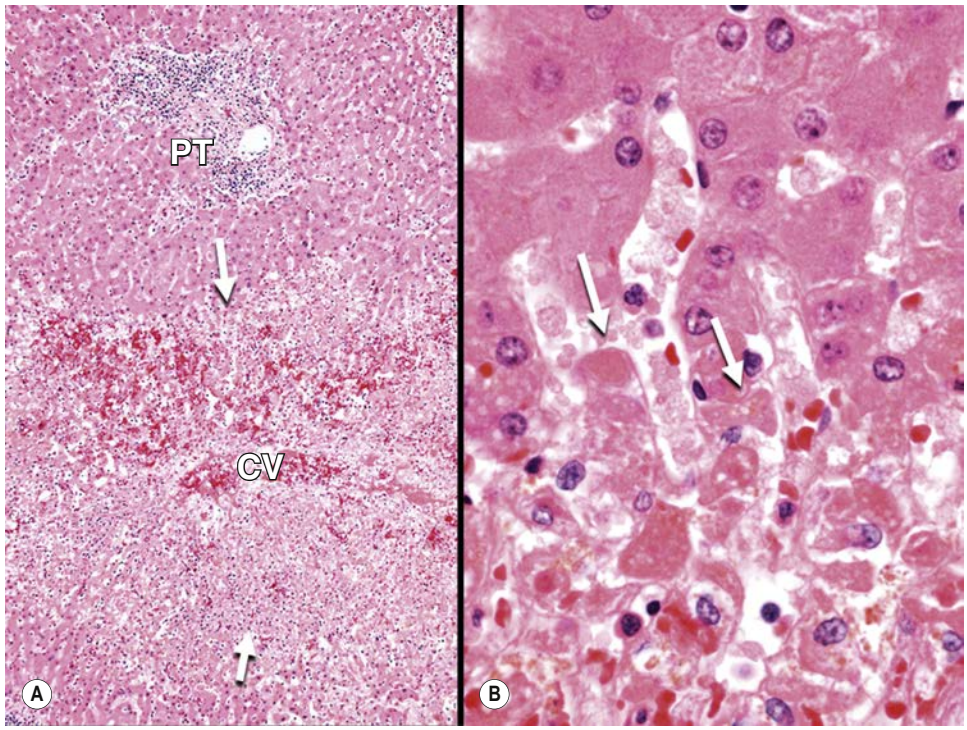

Fig. 12.12 Centrilobular congestion and coagulative necrosis. This patient died of cardiogenic shock. **A:** Congestion of the central vein (CV) and surrounding sinusoids reflects the mild right ventricular dilatation seen grossly at autopsy. Evidence of left-sided failure is noted between the arrows where there is also coagulative necrosis of hepatocytes. **B:** The intact mid-zonal hepatocytes at the top of this field show intact nuclei with preserved chromatin detail and nucleoli. By contrast, centrilobular hepatocytes (arrows) show coagulative necrosis with loss of nuclei, nuclear pyknosis, fragmentation and separation from the liver cords. (Postmortem liver, H&E). PT, portal tract.

Box 12.1 Histologic features associated with congestive hepatopathy
Centrilobular sinusoidal dilatation and/or congestion
Centrilobular hepatocyte cord narrowing and atrophy
Perivenular/perisinusoidal fibrosis (cardiac sclerosis)
Bridging fibrosis (central–central and/or central–portal)
Cirrhosis (cardiac cirrhosis), relatively uncommon
Periportal regenerative hyperplasia (variable)
Nodular regenerative hyperplasia
Extramedullary haematopoiesis (focal, variable, sometimes isolated sinusoidal megakaryocytes)
Periportal ductular reaction (variable)

obstruction[97] (**Fig. 12.13**). Periportal necrosis occurs rarely.[95] There may be regenerative hyperplasia of hepatocytes; chronic venous congestion is one cause of nodular regenerative hyperplasia (**Fig. 12.14**) and, very rarely, cirrhosis.[95] Perivenular and perisinusoidal fibrosis (cardiac sclerosis; **Figs 12.14 and 12.15**) reflect prior episodes of failure.[21,95] In some patients hepatocytes contain PAS-positive globules which probably represent phagosomes containing imbibed plasma proteins.[98] The globules are usually located in or near the congested areas. They can be distinguished from the globules of α_1-antitrypsin deficiency by their location and, if necessary, by immunochemical staining.

Liver biopsy plays a role in decision making for patients with severe cardiac disease who are being considered for heart transplantation versus combined heart–liver transplantation, particularly to assess the degree of hepatic fibrosis. Several simple scoring systems are available that provide semiquantitative scores for fibrosis in

congestive hepatopathy[99–101] (**Table 12.1**). A recognised interpretive problem in this area, however, is the known heterogeneity in histological findings in liver biopsies from individuals with advanced cardiac disease and venous outflow obstruction[102] (**Fig. 12.15**). Sampling error can therefore be substantial. Uncertainty with regard to the definitive stage of hepatic fibrosis should be taken into account in discussions with the clinical team; in such cases, other clinical or laboratory data may be critical to incorporate for appropriate patient management.[102]

The survival into young adulthood and beyond of individuals who previously underwent Fontan procedures for congenital heart disease with single ventricle pathophysiology (e.g. hypoplastic left heart syndrome) has raised surveillance concerns[103] in this unique population for progressive hepatic fibrosis and hepatocellular carcinoma,[104–108a,108b,108c] both of which show increasing risk with time elapsed since Fontan surgery. Centrilobular and perisinusoidal fibrosis are universal in these patients.[107,108b,108c] Interval hepatic ultrasonography and other methods for early detection of mass lesions are now recommended.[103,107–111]

Obstruction to large veins

Obstruction of the inferior vena cava or the main hepatic veins typically causes severe congestion. The many causes include thrombosis related to myeloproliferative disorders,[12,112] predisposing coagulopathies[113] and other haematological diseases. Disorders characterised by vasculitis, such as Behçet's disease, may be complicated by either venous outflow obstruction[114,115] or portal-vein obstruction.[116] An association of outflow obstruction with the use of oral contraceptives lacks conclusive proof.[117] Fibrous webs may represent a late consequence of thrombosis,[118,119] but there is some evidence to support an alternative, non-thrombotic pathogenesis.[120] Occasionally the obstruction results from administration of chemotherapeutic agents[12] or infection.[121] In some patients no cause can be discovered. While in Western countries primary hepatic vein thrombosis is more common than obliterative disease of the inferior vena cava (obliterative cavopathy), the reverse is true in the developing world.[122] Caval obstruction is often complicated by hepatocellular carcinoma.

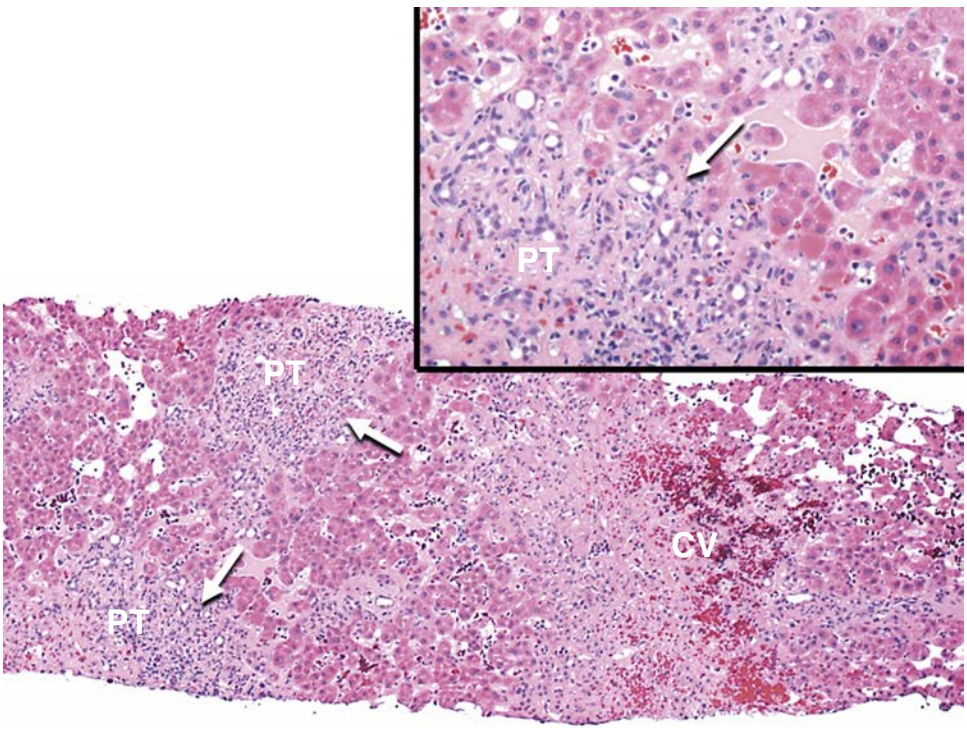

Fig. 12.13 Ductular reaction associated with congestive hepatopathy. This patient had long-standing biventricular failure with well-established fibrosis and chronic congestion affecting the centrilobular regions. The portal tracts (PT) show a prominent ductular reaction (arrows) although no clinical evidence of biliary obstruction was found in this patient. Inset: Higher magnification of the ductular reaction present in the PT. (Needle biopsy, H&E). CV, central vein.

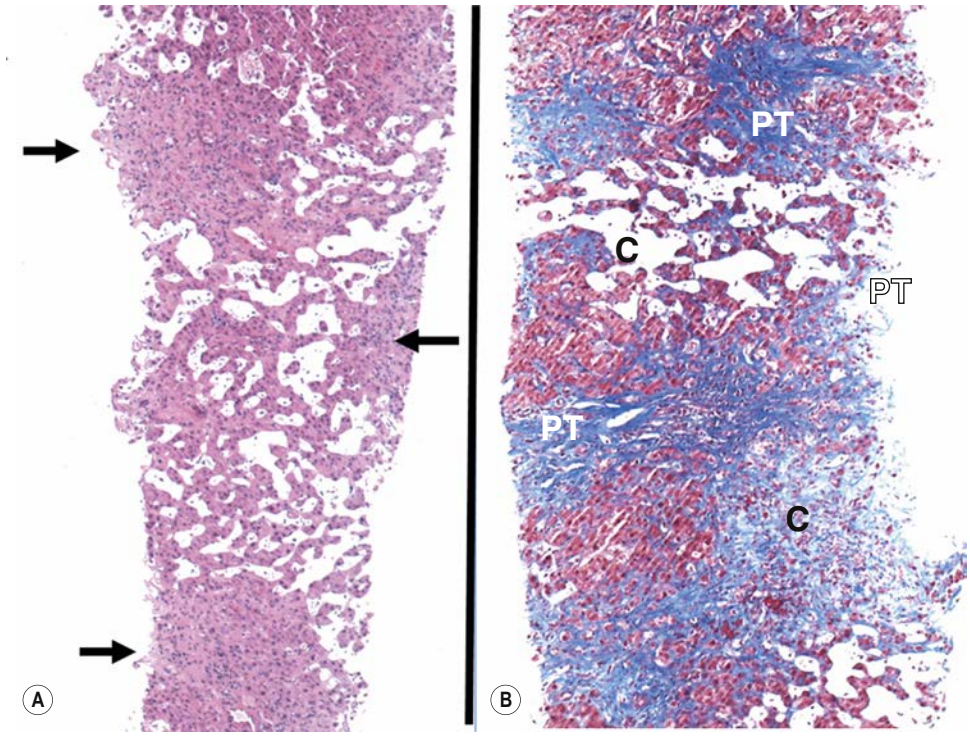

Fig. 12.14 Nodular regenerative hyperplasia with marked centrilobular sinusoidal dilatation and cardiac sclerosis. A: Long-standing biventricular failure with chronically elevated right heart pressures may result in sufficiently diffuse nodular regenerative hyperplasia of periportal regions (arrows) to cause portal hypertension with an associated increased hepatic venous gradient. **B:** The nodularity of this needle biopsy is associated with variable, but often marked cardiac sclerosis in centrilobular (C) regions, as well as bridging fibrosis to portal tracts (PT). (Needle biopsy, **A:** H&E, **B:** Masson trichrome stain.)

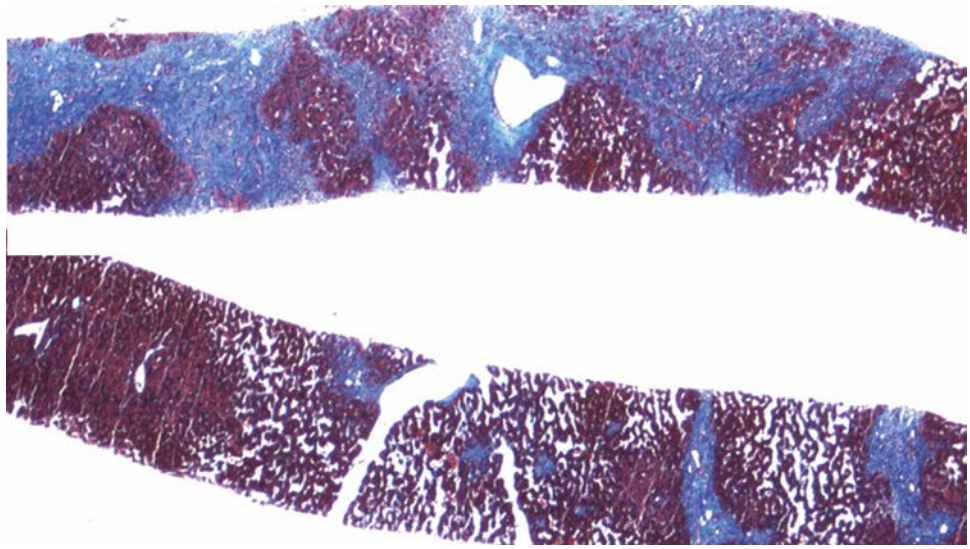

Fig. 12.15 Heterogeneity of hepatic histopathology in congestive hepatopathy and chronic hepatic venous outflow obstruction. This biopsy specimen, from a patient with long-standing cardiac disease who is listed for cardiac transplantation, was obtained in order to evaluate the possible need for combined heart–liver transplantation. The specimen consists of two needle cores of liver parenchyma with a striking discordance of morphological findings (the top core demonstrates Stage 3–4 fibrosis and nodularity tantamount to cirrhosis, while the bottom core shows merely centrilobular sinusoidal dilatation and no significant fibrosis). (Needle biopsy, Masson trichrome stain.)

In the acute stages much of the parenchyma may be replaced by blood. Sinusoids at the border between the haemorrhagic zones and the surviving parenchyma are dilated and empty (**Fig. 12.16**). Small efferent veins may be narrowed or blocked, depending on the cause of the obstruction (see the discussion on veno-occlusive disease (VOD) later). Portal-vein branches may also be thrombosed.[123] In acute or chronic venous outflow obstruction it is common to find normal-appearing portal tracts, but there may also be portal features that mimic biliary tract disease, including a ductular reaction, inflammation and portal and/or periportal fibrosis, typically unassociated with cholestasis.[97] Eventually the haemorrhage and congestion lead to fibrosis or even cirrhosis (**Fig. 12.17**). The pattern of cirrhosis following venous outflow obstruction is influenced by the presence or absence of concomitant portal venous thrombosis; this is associated with extensive portal–central–portal bridging, with presence of portal tracts within the fibrous septa.[26] In some cases parenchymal nodularity is due to nodular regenerative hyperplasia rather than true cirrhosis, and isolated nodules resembling focal nodular hyperplasia (**Ch. 11**) can develop as a result of locally increased arterial blood flow.[123]

Table 12.1 Simple fibrosis scoring system for congestive hepatopathy.

Stage of fibrosis/ cirrhosis	Distribution of fibrosis
Stage 1	Focal centrilobular fibrosis
Stage 2	Diffuse, multifocal centrilobular fibrosis
Stage 3	Bridging fibrosis (central–central; central–portal)
Stage 4	Cirrhosis

Fibrosis is often difficult to distinguish from simple acute condensation of pre-existing reticulin and collagen. Stains for elastic fibres are then sometimes helpful, as in the distinction between bridging necrosis and fibrosis (**Ch. 4**). Two further diagnostic problems should be noted. First, blocked veins may be missed in haematoxylin and eosin-stained sections so that a collagen stain should be examined if venous outflow obstruction is suspected. Thin-walled bypass channels should not be mistaken for patent veins. Second, the obstruction may not affect all the hepatic veins, so that parts of the liver escape serious congestion. As a result, biopsy samples may show considerable regional variability, which can lead to diagnostic confusion. It follows that a near-normal liver biopsy does not exclude a diagnosis of venous outflow obstruction.

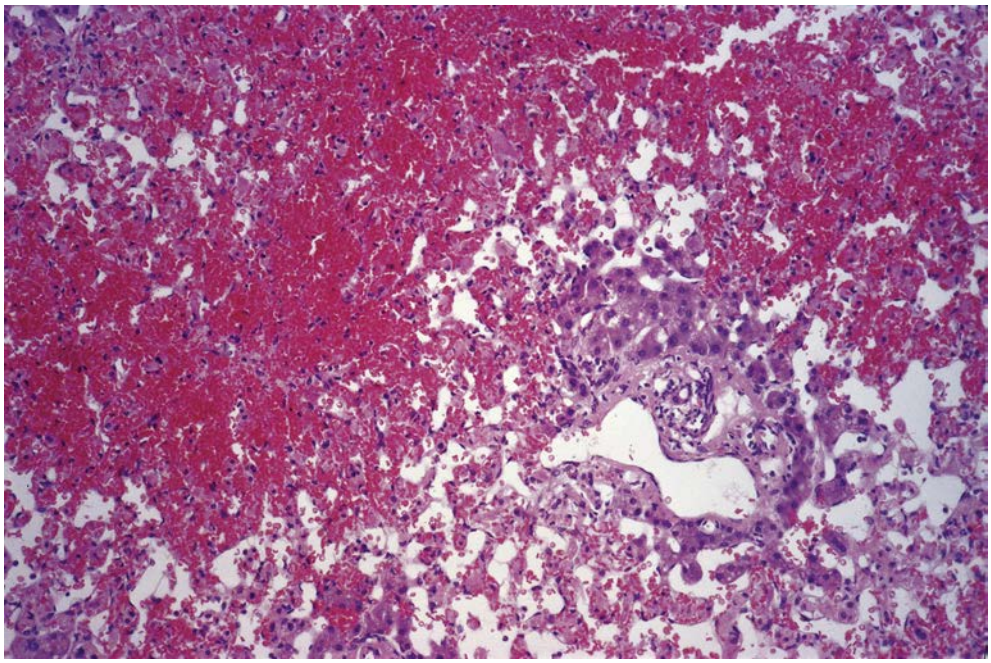

Fig. 12.16 Acute venous outflow obstruction. In this example, owing to obstruction of major veins (Budd–Chiari syndrome), much of the parenchyma has been replaced by blood. A few hepatocytes have survived around the portal tract (bottom right). (Wedge biopsy, H&E.)

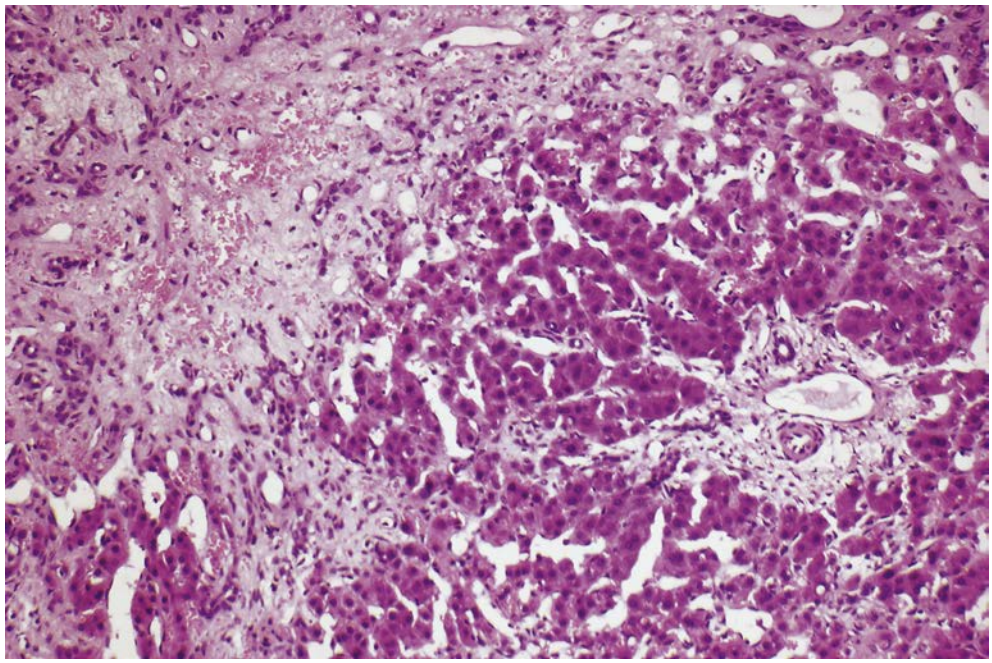

Fig. 12.17 Chronic venous outflow obstruction. Late in the disease fibrous tissue has been laid down in the congested areas, top left. Surviving parenchyma shows 'reversed lobulation' around a portal tract. (Needle biopsy, H&E.)

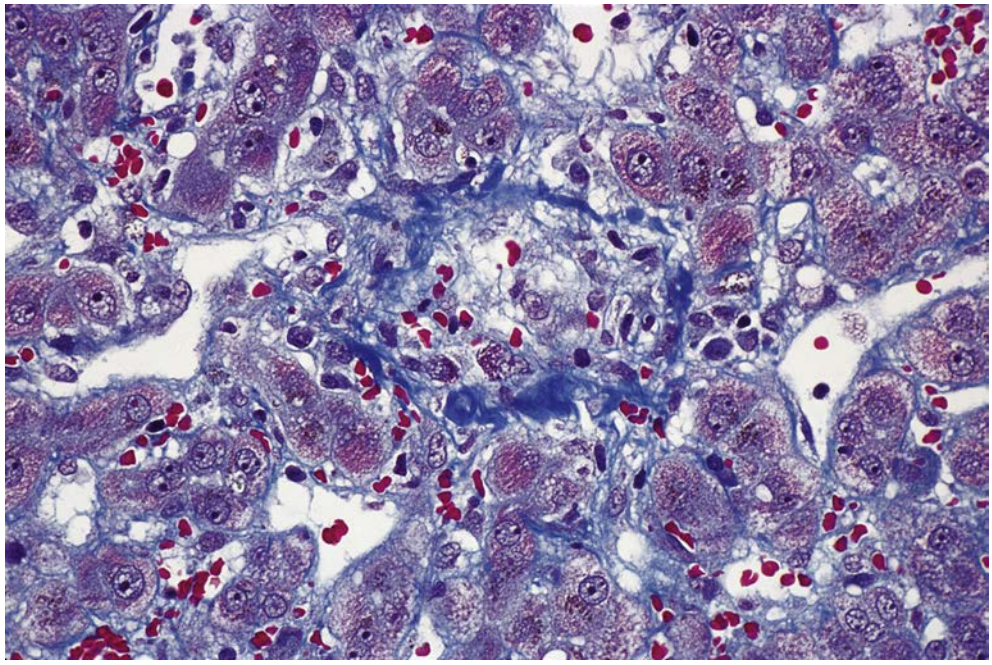

Fig. 12.18 Veno-occlusive disease. A terminal hepatic venule has been occluded by recently formed collagen and cells, following liver transplantation. (Needle biopsy, chromotrope–aniline blue.) (Section kindly provided by Professor AP Dhillon, London, UK.)

Sinusoidal obstruction syndrome/veno-occlusive disease

The recent term **sinusoidal obstruction syndrome** (SOS) has been used to describe the spectrum of sinusoidal congestion, dilatation, thrombosis and fibrous occlusion of efferent venules which developed due to injury to sinusoidal endothelial injury of varying aetiology, often chemical.[12,124–126] **Veno-occlusive disease (VOD)**—in which the smallest tributaries of the hepatic veins (i.e. the terminal hepatic venules and sublobular veins) are

occluded by fibrous tissue (**Fig. 12.18**)—is a manifestation of SOS. The venous lesions can thus be detected in needle biopsies. There is often associated fibrosis of sinusoidal walls (perisinusoidal fibrosis), probably mediated by hepatic stellate cells.[127] Apart from these lesions the changes are as for obstruction of large veins. As already noted, the borderline between obstruction to large and small veins is not sharp, because both may be affected by thrombosis, for example, in patients with coagulation disorders. Furthermore, thrombosis of large- or medium-sized hepatic veins may lead to fibrous intimal thickening of smaller vessels. Rarely, an exuberant form of fibrous obliteration involves veins of all calibres and has been shown to recur after liver transplantation.[128] Careful inspection of the congested sinusoids in SOS in liver biopsy specimens may disclose loss or discontinuity of endothelium from the vascular lining, endothelial and/or hepatocyte extrusion into the sinusoidal lumen and intravasation of red blood cells into the space of Disse (**see Fig. 16.23**).

As in the case of obstruction to larger veins, there are many causes of SOS and VOD. The classical cause, ingestion of pyrrolizidine alkaloids, remains a hazard.[129] Sinusoidal endothelium in general is also susceptible to similar toxic injury, particularly in individuals receiving chemotherapy or myeloablative conditioning therapy prior to haematopoietic cell transplantation.[12,130] Among these agents are 6-thioguanine, busulfan, gemtuzumab–ozogamicin and oxaliplatin.[12] Venous occlusion is also seen following renal or liver transplantation.[100,104–133] Other associations and causes are irradiation of the liver,[134] AIDS,[135] heroin addiction,[51] primary vascular disease,[136] Hodgkin's disease,[137,138] drug therapy[75,139] and arsenic poisoning.[140] Epithelioid haemangioendothelioma of the liver can mimic VOD because of the characteristic vascular occlusions produced by tumour invasion, and can also give rise to clinical and histological features of the Budd–Chiari syndrome.[141] Finally, careful examination of connective tissue stains may reveal occluded veins in the livers of patients with alcoholic and non-alcoholic fatty liver disease[142,143] and indeed in cirrhosis from any cause.[144]

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CHAPTER

13 Childhood Liver Disease and Metabolic Disorders

Introduction

Paediatric liver biopsies present a unique set of diagnostic problems for the pathologist,[1] many of which become clinically apparent in the first few months of life as **neonatal cholestasis**.[2] Among the important disorders one must consider in evaluating neonatal liver biopsies are **extrahepatic biliary atresia**, **paucity of intrahepatic bile ducts** (syndromatic and non-syndromatic types), **metabolic diseases**, **viral hepatitis** and the hepatic effects of **parenteral nutrition** (Table 13.1). Common to many of these conditions are the histological features of **cholestasis** and **giant-cell hepatitis** (formation of multinucleated hepatocytes). Because these features are not specifically diagnostic of any one neonatal liver disease, the pathologist must be acquainted with other biopsy changes by which to establish or suggest the diagnosis. In many instances, assays of metabolic enzymes and products in serum and liver tissue take diagnostic precedence over routine histopathological interpretation. Electron microscopy may be required to assess the structure of organelles or storage material in hepatocytes or Kupffer cells, particularly when lysosomal storage disorders are being considered. Consultation with investigators dealing with mitochondriopathies,[3,4] mutations of bile-salt transport proteins[5] and expression of proteins involved in blood vessel and bile-duct morphogenesis (e.g. Jagged proteins and Notch receptors) should be considered if special studies are needed to determine the cause of neonatal cholestasis. Childhood liver tumours are discussed in **Chapter 11**.

Diagnostic approach to neonatal liver biopsy

Histopathological examination of neonatal liver biopsies may benefit from a systematic checklist of questions by which the major diagnostic concerns in neonatal liver disease can be evaluated. A simplified, stepwise set of seven questions can be asked:

1. *Is the acinar structure normal for age?* As described in **Chapter 3**, the hepatic plates are two cells thick until 5 or 6 years of age and should not be misconstrued as a pathological change. As with adult biopsies, the presence of fibrosis, nodularity or cirrhosis should be noted early in the biopsy evaluation and correlated with other histological features which may define the aetiology.
2. *Are cholestasis and giant cells present?* As indicated earlier, neither of these is diagnostically specific. If present, the next interpretive steps should be examination of portal tracts for evidence of biliary tract obstruction (e.g. atresia) and of portal tracts and parenchyma for evidence of hepatitis.

Table 13.1 Liver biopsy interpretation in neonatal cholestasis.*

Aetiology	Histological features
Extrahepatic biliary atresia	Ductular reaction; ductular cholestasis; portal and periportal fibrosis
Paucity of intrahepatic bile ducts	Loss of interlobular bile ducts (bile duct-to-hepatic artery ratio < 1)
Neonatal hepatitis	Portal and lobular mononuclear cell inflammation; apoptotic bodies
Metabolic disorders	Steatosis; fibrosis or cirrhosis; storage product in liver cells or Kupffer cells (see specific disorder)
Parenteral nutrition	Ductular reaction; portal fibrosis or cirrhosis

*Many of the conditions shown in **Table 13.1** are associated with histological cholestasis and formation of giant multinucleated hepatocytes, in addition to the diagnostic features listed.

3. *Are histological changes of hepatitis present?* Mononuclear cell infiltrates within acini and portal tracts associated with liver-cell degeneration should be sought when considering cytomegalovirus, Epstein–Barr virus, rubella or hepatitis virus infections.
4. *Are the interlobular bile ducts normal?* This question has three major ramifications. **Abundance of bile ducts** usually signifies some form of biliary obstruction, such as extrahepatic atresia or choledochal cyst. **Paucity of bile ducts** (**ductopenia**, **vanishing bile-duct syndrome**) may be due to developmental, metabolic or infectious causes. Lastly, **malformations of bile ducts** comprise a spectrum of problems related to abnormal remodelling of the embryonic bile-duct plate (fibropolycystic diseases).
5. *Does the biopsy specimen contain iron or copper?* Although rare, neonatal haemochromatosis[6] and Indian childhood cirrhosis (copper toxicosis in young children) are serious liver diseases with high mortality rates that must be excluded. In the older child and adolescent, Wilson's disease (**Ch. 14**) must not be overlooked. It should be noted, however, that fetal and neonatal liver contains much higher copper levels than that of adults, with an irregular tissue distribution.[7] Mild siderosis is also within the spectrum of normal findings in the fetal and neonatal liver.[8]
6. *Has the biopsy specimen been studied by diastase–periodic acid–Schiff (PAS) or immunoperoxidase staining to exclude α_1-antitrypsin deficiency?* The expression of α_1-antitrypsin deficiency is variable, and biopsies may not show diagnostic staining of retained enzyme within liver cells prior to 13–15 weeks of age. This condition should be histologically excluded whenever possible.
7. *Are storage cells present?* Abnormal storage products in liver cells or Kupffer cells may be seen in various metabolic diseases which cause hepatomegaly and failure to thrive. These should be sought on routine haematoxylin and eosin (H&E) as well as special stains.

Neonatal hepatitis

Inflammation and hepatocellular damage in the neonatal period may result from infections, from inborn errors of metabolism and from primary disorders of immune dysregulation. Infections include type B hepatitis, cytomegalovirus infection and rubella among others; inborn errors of metabolism include α_1-antitrypsin deficiency, galactosaemia and bile-acid synthetic defects.[9] Immune dysregulatory conditions include autoimmune

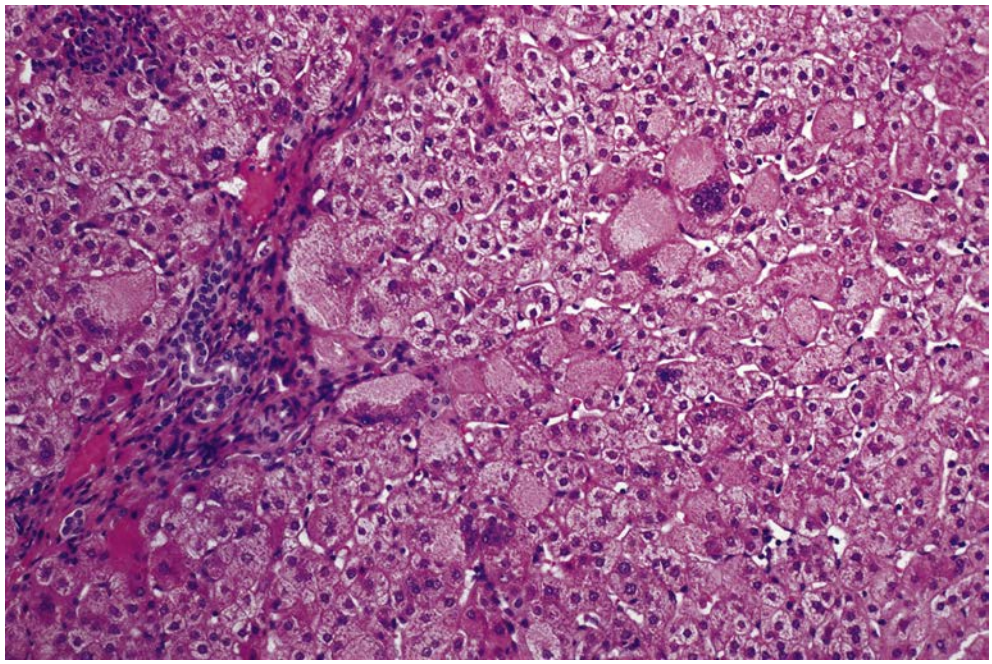

Fig. 13.1 Neonatal (giant-cell) hepatitis. The parenchyma consists of multinucleated giant liver cells and the portal tract shown is infiltrated by lymphocytes. (Wedge biopsy, H&E.)

hepatitis (AIH), as well as rare disorders affecting specific lymphoid cell populations. An example of the latter is the unusual case of paediatric acute liver failure (PALF), in which overrepresentation of CD103-positive Trm (resident memory T cells) and CD8 (cytotoxic T cells) can result in a severe hepatitis and acute liver failure.[10] Disorders at the ultrastructural or molecular level may also need to be considered, such as neonatal hepatitis due to depletion of mitochondrial DNA.[11–17] A diagnosis of neonatal hepatitis is therefore a signal for further investigation. The histological picture is broadly similar whatever the cause. There is a variable degree of hepatocellular swelling and multinucleation, cholestasis and portal inflammation (**Fig. 13.1**). Lobular inflammation may be mild. Liver-cell necrosis and swelling result in collapse and distortion of the reticulin framework. Fibrosis is sometimes already well developed, as in neonatal haemochromatosis (**Ch. 14**) or the severe perinatal liver disease which may rarely be seen in Down's syndrome (**Chapter 15**).[18] Giant multinucleated hepatocytes are commonly seen, whatever the cause of the hepatitis (**Fig. 13.2**). The outcome of neonatal giant-cell hepatitis is resolution, liver failure, cirrhosis or a chronic cholestatic course. The variety of different outcomes is well illustrated in α_1-antitrypsin deficiency.[19]

From a histological point of view, the main differential diagnosis of neonatal hepatitis is extrahepatic biliary obstruction, which may require surgical treatment. Giant multinucleated hepatocytes, an altered reticulin structure and little or no ductular reaction are more prominent in hepatitis than in biliary obstruction, while cholestasis is usually more severe in atresia and there is typically a ductular reaction.

Extrahepatic biliary atresia

Extracellular biliary atresia (EHBA) results from inflammation and destruction of all or part of the extrahepatic bile-duct system *in utero* or in the perinatal period.[20] Pathological studies of atretic bile-duct segments[21–24] show chronic inflammation and obliterative fibrosis, sometimes with a few remaining bile-duct cells[25] seen on routine stains or cytokeratin immunostaining. Satisfactory bile drainage and an improved outcome after the Kasai

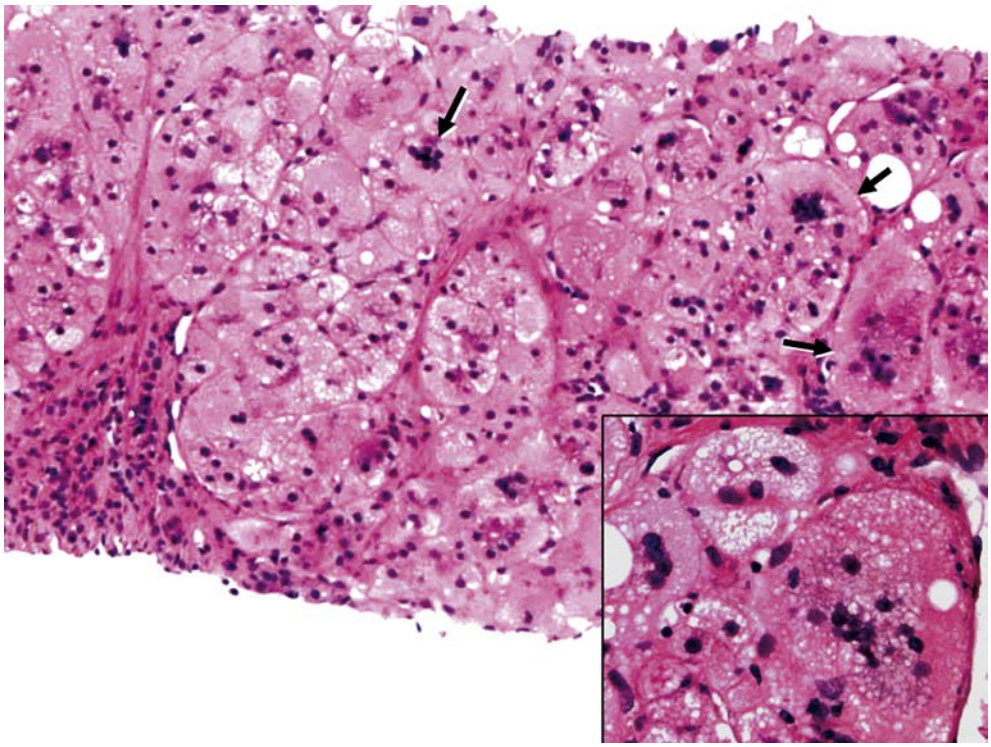

Fig. 13.2 Neonatal (giant-cell) hepatitis. Multinucleated giant hepatocytes are present (arrows), and there is mild parenchymal disarray and inflammation. The portal tract at left shows mild periportal fibrosis and inflammation. Hepatocytes contain finely divided lipid vacuoles (microvesicular steatosis), as seen in the inset. This neonate also had neurological deficits and genetic analysis demonstrated a mitochondriopathy associated with mitochondrial DNA depletion. (Needle biopsy, H&E.)

portoenterostomy[26] have sometimes been associated with identification of bile ducts with lumens of 150 μm or greater at the proximal resection margin.[22] Optimal surgical results are obtained if the Kasai procedure (hepatic portoenterostomy) is performed within the first 8 weeks of life,[27] with approximately 30% surviving into adulthood with their native liver.[28] Many patients, nevertheless, later require liver transplantation.[29,30]

The pathogenesis of the destructive process in extrahepatic atresia remains speculative,[30] but considerations have included viral infections (reovirus type 3, rotavirus), exposure to toxins (e.g. outbreaks of biliary atresia in Australian livestock from ingestion of the plant isoflavonoid biliatresone[31]), abnormalities in regulatory T cells,[32] abnormal remodelling of the embryonic bile-duct plate and disorders of Jagged protein/Notch receptor and Hedgehog signalling.[20,33] DNA microarray studies suggest a gene profile of abnormal cell signalling and transcription regulation.[34] There may be associated congenital abnormalities, including polysplenia,[35] intrahepatic biliary cysts,[36] laterality defects and cardiovascular, musculoskeletal and genitourinary defects[37,38] in approximately 20% of cases with more severe and earlier disease (the 'embryonic' form of biliary atresia). Heterozygous missense mutations in the *CFC1* gene, a determinant gene for left–right axis, have been identified in some BA polysplenia syndrome patients.[39] By contrast, the majority of patients have the 'perinatal' form without such anomalies. Expression of various regulatory genes appears to differentiate the two types.[40] The process of biliary atresia is a dynamic one which may also involve the intrahepatic bile ducts,[41,42] and result in progressive fibrosis[43] even after Kasai surgery.[44]

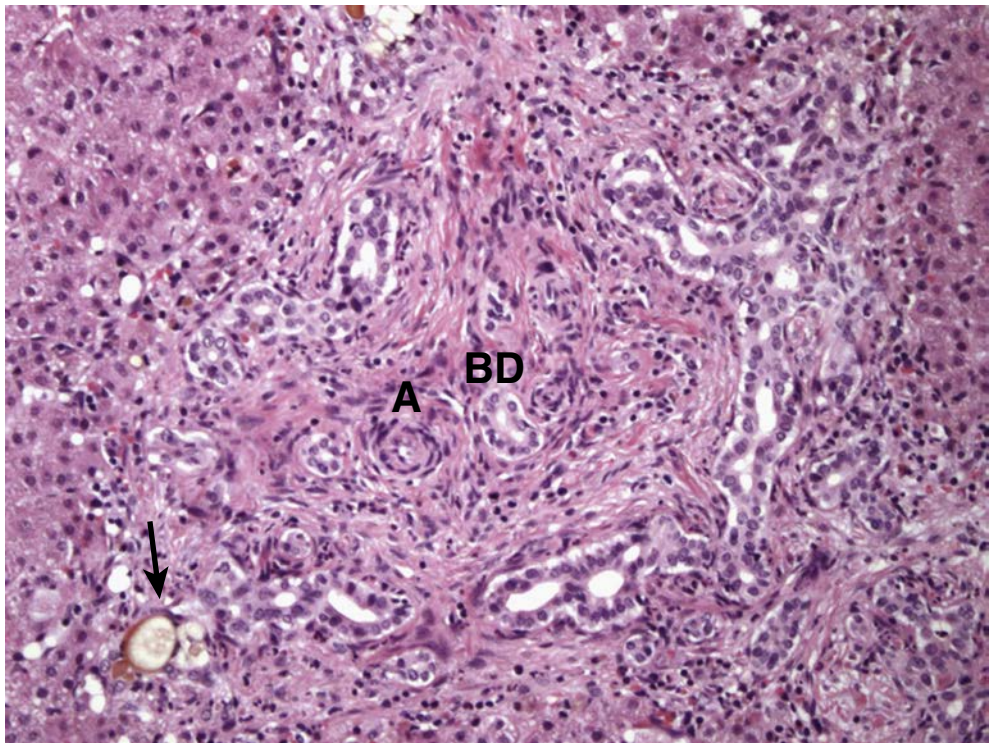

Fig. 13.3 Extrahepatic biliary atresia. The portal tract shows the characteristic diagnostic changes of fibrosis and prominent ductular reaction, with numerous proliferated bile ductular structures circumferentially surrounding the tract. Focal bile ductular cholestasis is evident (arrow). Note the intact hepatic arteriole (A) and similar-calibre bile duct (BD) at the centre of the tract. (Needle biopsy, H&E.)

Liver biopsy shows cholestasis and portal tract changes resembling those of large bile-duct obstruction in the adult (Ch. 5). Portal tracts are enlarged by oedema and fibrosis (which varies depending on the age at biopsy), a striking ductular reaction and infiltrating neutrophils with fewer chronic inflammatory cells (**Fig. 13.3**). Native interlobular bile ducts are usually intact and can be identified near hepatic arterioles. Bile-containing portal macrophages are often also present. Ductular structures may contain inspissated bile (ductular cholestasis; **Fig. 13.3**) and occasionally resemble the embryonic bile-duct plate described by Jörgensen[45] (**Figs 13.4 and 13.5**). A prominent ductular reaction is the major histological point of distinction from neonatal hepatitis.[46] There is panlobular cholestasis with accentuation in zone 3. Giant cells are common, but not as numerous or as striking as in neonatal hepatitis. The lobular architecture remains intact, except in patients diagnosed late in the disease who may then show secondary biliary cirrhosis (**see Fig. 5.11**). Many histologic parameters have been evaluated in previous studies in order to determine the key predictors of EHBA.[47] The most recent investigation of infants with cholestasis enrolled in a study sponsored by the Childhood Liver Disease Research Network (CHiLDReN) found that *bile plugs in portal bile ducts/ductules* and *portal stromal oedema* were the strongest independent histologic predictors of obstruction.[48] The study also found five histologic features that were independent predictors of EHBA by multivariate analysis (**Box 13.1**). Portal fibrosis is a histological parameter that worsens with age, so that higher stages (stage 3–4) are noted in older infants. It should be kept in mind that in this large-scale study, 10% of biopsies of proven EHBA cases lacked features of biliary obstruction.

Box 13.1 Five histological predictors of extrahepatic biliary atresia (EHBA)[48]

- Bile plugs in bile ducts/ductules*
- Portal stromal oedema*
- No bile duct paucity
- Absent to rare giant-cell transformation
- Absent to rare extramedullary haematopoiesis (EMH)

**Strongest independent histological predictors of EHBA.*

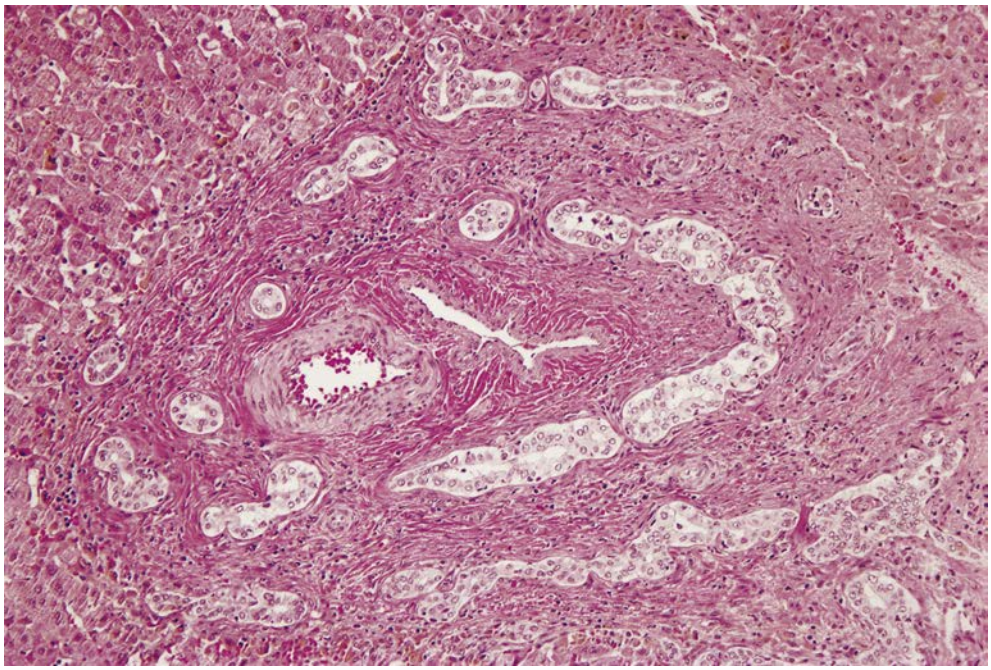

Fig. 13.4 Extrahepatic biliary atresia with bile-duct plate-like structures. The proliferating bile-duct structures in this case resemble the embryonic bile-duct plate. (Wedge biopsy, H&E.)

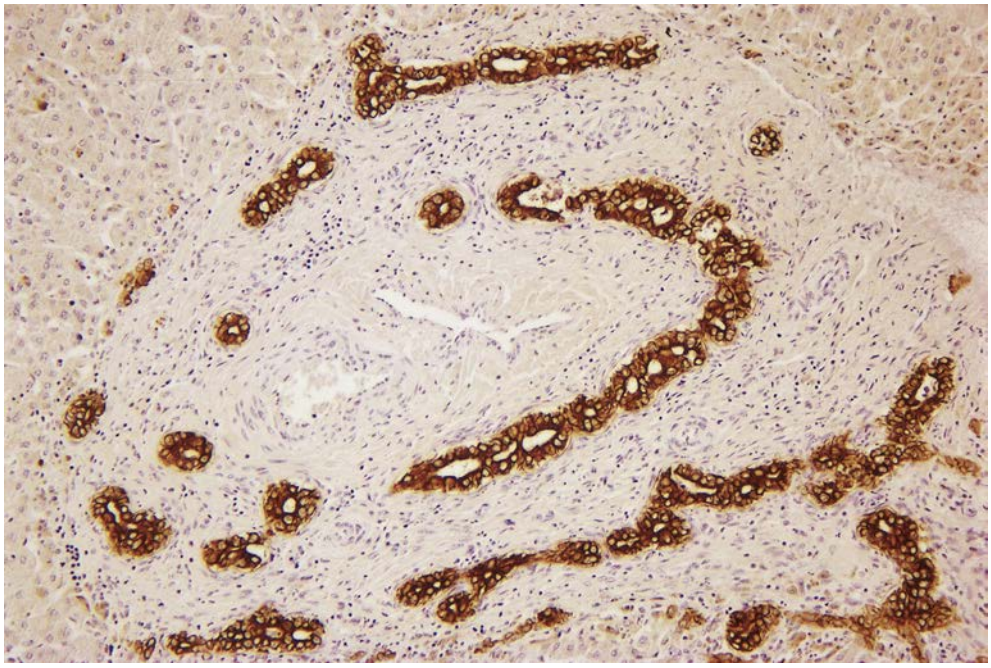

Fig. 13.5 Extrahepatic biliary atresia with bile-duct plate-like structures. The field shown in **Fig. 13.4** stained with antibodies to cytokeratin highlights the circumferential portal bile-duct structures resembling the embryonic bile-duct plate. (Wedge biopsy, specific immunoperoxidase.)

Alpha$_1$-antitrypsin deficiency and total parenteral nutrition cause identical changes to EHBA[47] and therefore require appropriate exclusion before rendering a pathological diagnosis of biliary atresia.

Choledochal cyst

Although rare, these cystic lesions of the extrahepatic biliary tree should be considered in the differential diagnosis of paediatric jaundice (and rarely in adults).[49,50] If liver biopsy is undertaken, histologic features of acute and chronic bile-duct obstruction are present, but these do not define the site or cause of obstruction; biliary atresia is a major differential diagnosis in this setting. Five types of choledochal cyst (CCs) are recognised radiologically.[49] Excision is the treatment of choice. Resected cysts show lining epithelium overlying a stromal connective tissue layer rich in smooth muscle actin-positive myofibroblasts. This histology is distinct from the occasional cyst seen in biliary atresia duct remnants, which show a thick, compact collagen layer facing the cyst lumen, without overlying epithelium.[51]

Autoimmune sclerosing cholangitis

This hybrid condition affecting children and adolescents combines the cholangiographic and cholestatic abnormalities of sclerosing cholangitis with prevailing autoimmune features, including positive serum anti-nuclear and/or smooth-muscle antibodies, elevated serum gamma globulin, abnormal serum aminotransferases and the histopathology of AIH (i.e. lymphoplasmacytic portal inflammation with interface hepatitis).[52] A prospective study from King's College Hospital, London, UK, found inflammatory bowel disease in 44% of the autoimmune sclerosing cholangitis (ASC) patients. Biopsy features can vary over time, toggling between the hepatitic features of AIH on one occasion, biliary features and the progressive periductal fibrosis of primary sclerosing cholangitis on another, or both, regardless of the presence of biliary lesions on cholangiography (**Fig. 13.6**). Current guidelines recommend cholangiography as part of the workup of children with autoimmune liver disease in order to exclude ASC.[53a]

Langerhans cell histiocytosis

Langerhans cell histiocytosis (LCH), a clonal proliferative disorder of CD1a/CD207 dendritic cells, may present with isolated liver involvement or can be part of a multisystem disease involving several organs. The peak incidence is in children 1 to 4 years of age (the mean age of diagnosis in adults is 30 years, although the disease may be overlooked and only diagnosed years later).[53a,53b,53c] The main presentations of liver involvement by LCH are sclerosing cholangitis, bile duct paucity or, later, biliary cirrhosis (**Fig. 13.7**). Infiltrating Langerhans cells with ample pink cytoplasm and kidney-bean- or boomerang-shaped vesicular nuclei with small nucleoli are seen individually or in granuloma-like clusters within hepatic sinusoids as well as in portal tracts and can be identified with Langerin, S-100 and CD1a immunostains. The bile-duct lesions show periductal fibrosis and oedema with surrounding acute and chronic inflammatory cells and variable numbers of Langerhans cells.

Paucity of intrahepatic bile ducts in childhood

Two varieties of intrahepatic bile-duct paucity (formerly called intrahepatic biliary atresia) are recognised in childhood: **syndromatic** and **non-syndromatic**.[54] In syndromatic paucity[55,56] (Alagille's syndrome or ALGS, arteriohepatic dysplasia), loss of small intrahepatic bile ducts is associated with abnormal facies, vertebral anomalies and various other malformations. The pathogenesis is linked to mutations in the *Jagged 1* gene (*JAG1* on

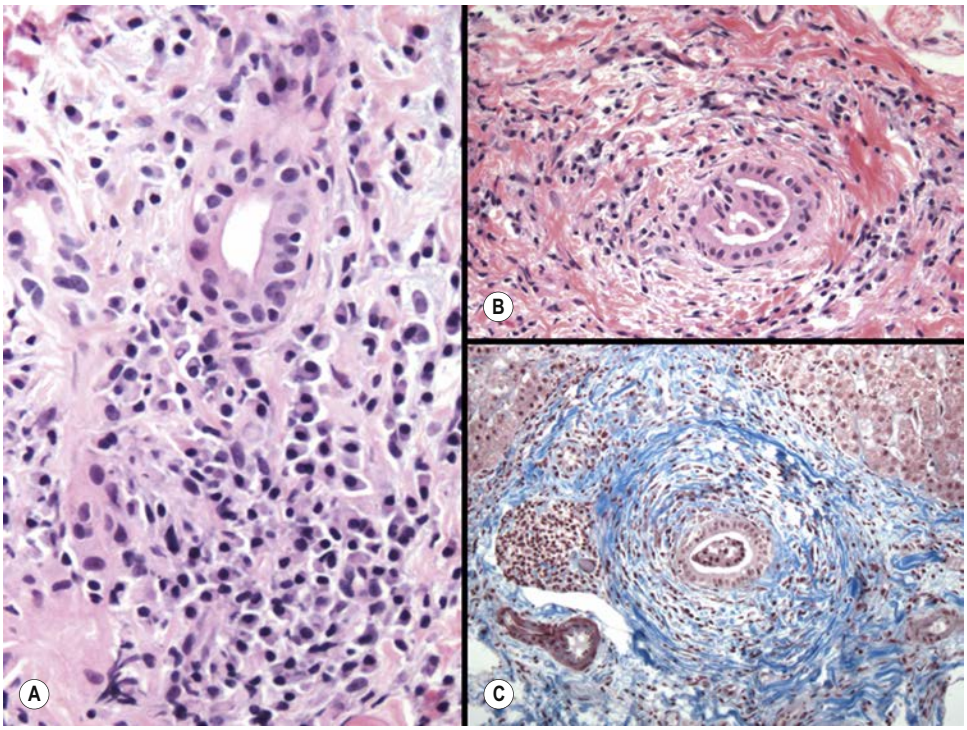

Fig. 13.6 Autoimmune sclerosing cholangitis. A: The portal tract is densely infiltrated by plasma cells which partially surround (and focally infiltrate) the interlobular bile duct. **B:** An 'onion skin' pattern of oedematous periductal fibrosis is present and resembles that seen in primary sclerosing cholangitis (PSC), but the degree of plasma cell-rich inflammation also surrounding the duct reflects the additional features of autoimmune hepatitis. **C:** PSC-like onion-skin fibrosis combined with excessive lymphoplasmacytic infiltrate resembling autoimmune hepatitis. (Needle liver biopsy; **A** and **B:** H&E, **C:** Masson trichrome.)

20p12.2) that produce a structurally abnormal ligand for binding in the Notch signalling pathway which is involved in cell–cell interactions in differentiation and the development of intrahepatic bile ducts.[57–59] Recent data have shown that 94% of cases with phenotypic features of ALGS have *JAG1* mutations and a small number show mutations for its receptor Notch2.[60,61] There is associated impairment of branching and elongation of hilar ducts distally into the liver periphery.[62] Increased mortality in these patients is linked to the presence of intracardiac congenital heart disease.[63] In non-syndromatic paucity, duct loss is not associated with facial or other anomalies. In some patients it may be related to a definable cause such as α_1-antitrypsin deficiency or cytomegalovirus infection,[64] while in others there is no detectable aetiological factor. The exact time of onset of bile-duct injury is difficult to establish accurately and probably varies from case to case. Some patients have active destruction of ducts in the first few weeks of life[56] and later stabilise, potentially with few symptoms or only mild chronic cholestasis, into young adulthood. In others, cirrhosis and liver failure may develop within months or many years later.[65] It has been speculated that there may be a small subgroup of patients with non-syndromatic paucity in which cholestatic disease first presents in adulthood[65] (**idiopathic adulthood ductopenia**).[66]

Histologically, in both forms of intrahepatic duct paucity there is canalicular cholestasis and chronic periportal cholestasis. Portal tracts show a variable degree of fibrosis and small bile ducts are scanty or absent[67] (**Fig. 13.8**). Step sections and cytokeratin 7 or 19 immunostaining may be needed for thorough assessment of duct numbers which, as in primary

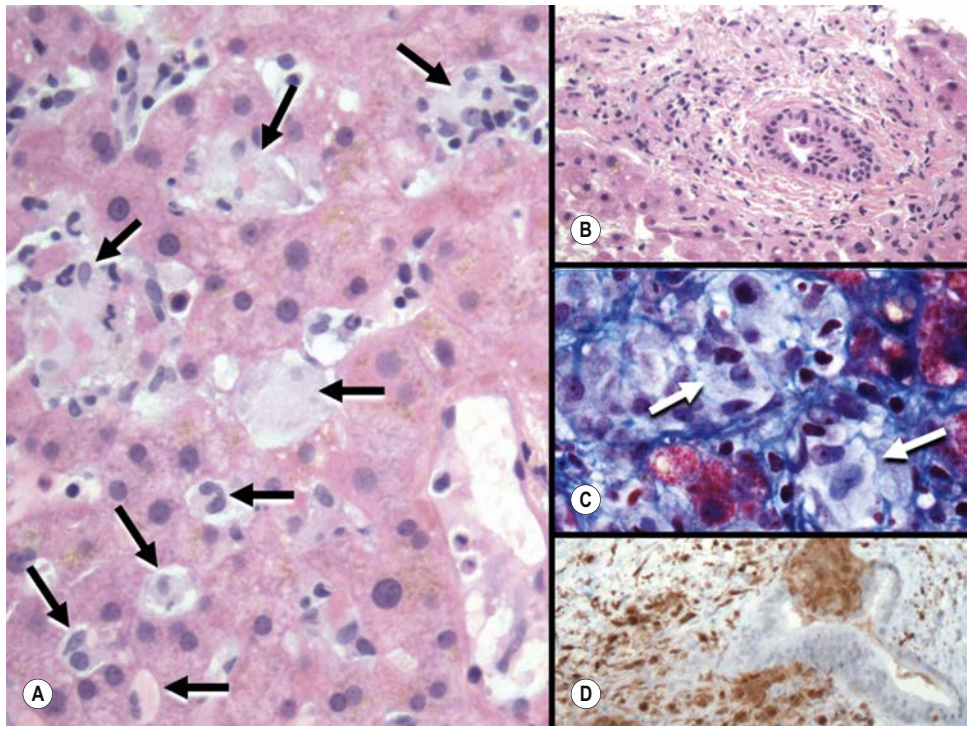

Fig. 13.7 Langerhans cell histiocytosis involving the liver, with evolving features of sclerosing cholangitis. A: Langerhans cells (arrows) with ample pink cytoplasm and 'kidney-bean'-shaped nuclei and prominent nucleoli infiltrate the lobular sinusoids, either singly or in small clusters that resemble microgranulomas. **B:** Periductal fibrosis is developing. **C:** Clusters of Langerhans cells (white arrows) are readily seen within sinusoids on trichrome stain. **D:** Langerin immunostain shows numerous infiltrating Langerhans cells with focal extension into the bile duct. (Needle biopsy; **A** and **B:** H&E; **C:** Masson trichrome; **D:** Specific immunoperoxidase.)

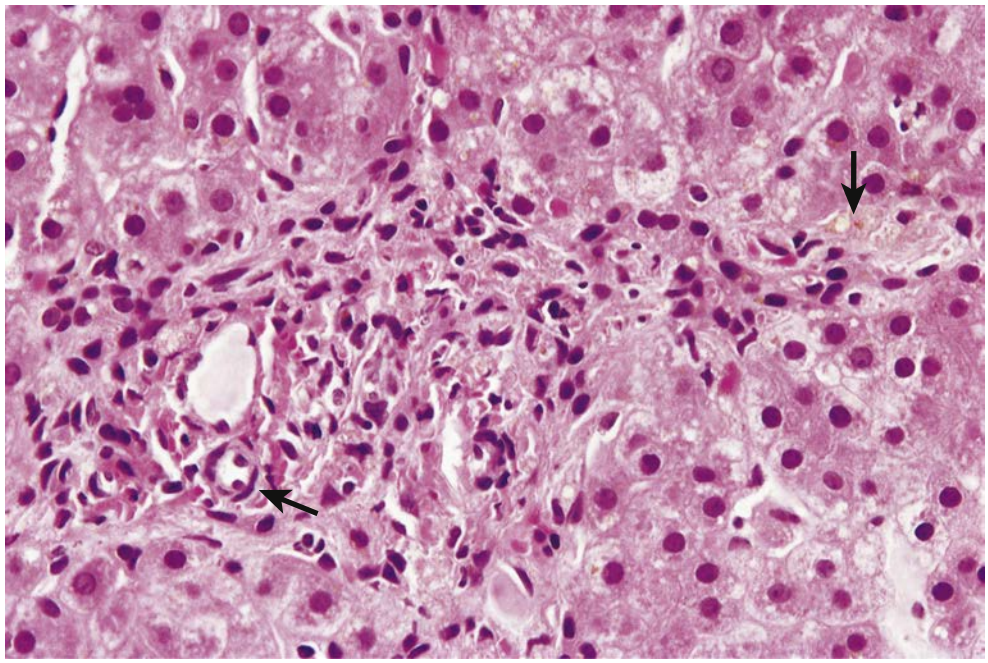

Fig. 13.8 Paucity of bile ducts in childhood. The portal tract shows an artery (left arrow) but no corresponding bile duct of similar calibre. There is periportal cholestasis (right arrow). (Needle biopsy, H&E.)

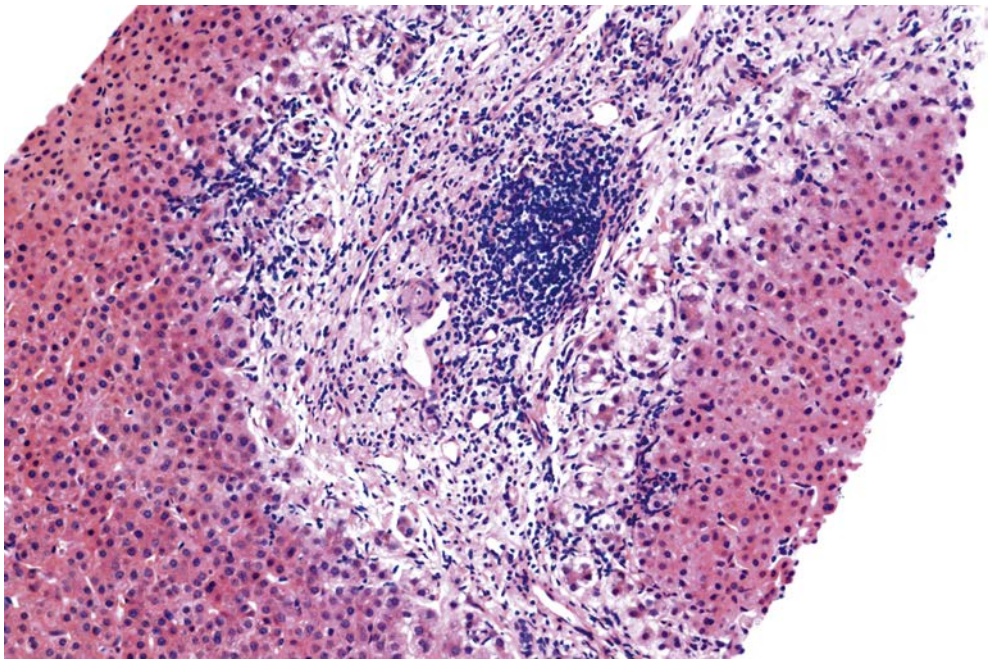

Fig. 13.9 Paucity of bile ducts in childhood. A lymphoid aggregate is present at the former site of the bile duct. (Needle biopsy, H&E.)

biliary cirrhosis, should approximately correspond to the number of arteries of similar size. A ductular reaction is usually not a prominent feature, in contrast to extrahepatic biliary atresia.[68] Immunohistochemical staining for clusters of differentiation marker CD10 (neutral endopeptidase) is normally identified on bile canaliculi, but is absent before the age of 24 months and also in Alagille's syndrome,[69] which can be helpful when used in the appropriate clinical setting. Inflammation is often slight or even absent, but lymphoid aggregates may be seen in the place of bile ducts (**Fig. 13.9**). Secondary biliary cirrhosis develops in some patients.[65,70] Alpha$_1$-antitrypsin deficiency should be looked for in all patients with paucity of ducts. Duct paucity has also been described in association with LCH.[71,72] As primary sclerosing cholangitis can also present in childhood,[73] it should be considered in the differential diagnosis.

Fibropolycystic diseases

The term **fibropolycystic diseases** covers a number of congenital abnormalities involving bile ducts, many of them related to an abnormal remodelling of the embryonic 'bile-duct plate'.[74–84] They include congenital hepatic fibrosis, Caroli's disease (congenital dilatation of the intrahepatic bile ducts), microhamartoma (von Meyenburg complex), choledochal cyst,[85] and both infantile and adult forms of polycystic disease. The first four of these carry an increased risk of carcinoma of the biliary tree.[86–89] The bile-duct plate, first seen at approximately 8 weeks of gestation, is a layer of primitive small cells encircling the portal tract mesenchyme (**Figs 13.4 and 13.5**). Progressive involution of most of these cells, with acquisition of strong cytokeratin 7 and 19 positivity in those remaining, is the process by which mature interlobular bile ducts of the portal tracts are formed.[75–78] Persistence of portions of the ductal plate and abnormal remodelling (the 'ductal plate malformation' described by Jörgensen[45]) lead to ectatic and irregularly shaped bile ducts set in dense fibrous stroma, the basic histopathological feature common to all fibropolycystic diseases. Mutations in genes encoding proteins found on primary cilia of bile-duct epithelium and resultant ciliary defects in mechanical, chemical and osmotic sensing underlie the characterisation of many of these diseases as **'ciliopathies'**.[79–82]

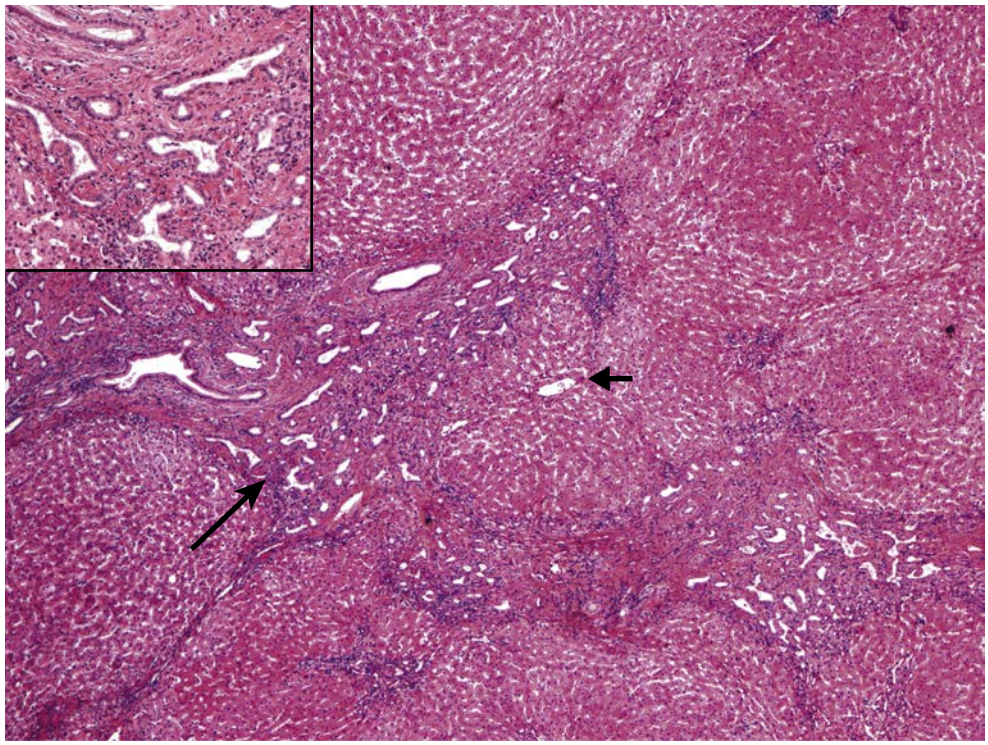

Fig. 13.10 Congenital hepatic fibrosis. Several portal tracts are interconnected by bridging fibrous septa containing ductal-plate malformations. The fibrosis surrounds normal parenchyma with a terminal venule (short arrow) preserved in a central position. Inset: Higher magnification of the abnormal duct structures seen at lower left (long arrow). (Explant liver, H&E.)

Congenital hepatic fibrosis

Congenital hepatic fibrosis is a recessively inherited condition, which presents as hepatomegaly or the effects of portal hypertension, usually in childhood but occasionally in adults.[90] Some cases have been associated with phosphomannose isomerase deficiency in which the resultant hypoglycosylation may affect remodelling of the bile-duct plate.[91] The liver is enlarged and very hard. Islands of normal liver parenchyma with unaltered vascular relationships are separated by broad and narrow septa of dense, mature fibrous tissue containing elongated or cystic spaces lined by regular biliary epithelium (**Fig. 13.10**). These represent cross-sections of the hollow structures constituting the ductal plate malformation. Two separate sets of duct-like structures can often be identified, one lying centrally in the septa, the other near the parenchyma. The lumens may contain inspissated bile. Portal-vein branches are small and inconspicuous in some cases. There is usually no cholestasis, necrosis, inflammation or hepatocellular regeneration. In older patients with congenital hepatic fibrosis, the abnormal duct-like structures may be less apparent because of atrophy.

Congenital hepatic fibrosis must be differentiated from cirrhosis, in which there is nodular regeneration and often inflammation and necrosis, and in which the abnormal biliary channels are not seen. The shape of the parenchymal islands in congenital hepatic fibrosis is very similar to that seen in secondary biliary cirrhosis (**see Fig. 5.11**). In this condition the septa contain irregular, newly proliferated bile ducts rather than congenitally abnormal plates; the connective tissue of the septa is loose and inflamed and there may be cholestatic features. Histological cholangitis, other types of inflammation or cholestasis in a liver with the characteristic features of congenital hepatic fibrosis should raise the possibility of coexisting Caroli's disease. The combination constitutes Caroli's syndrome.

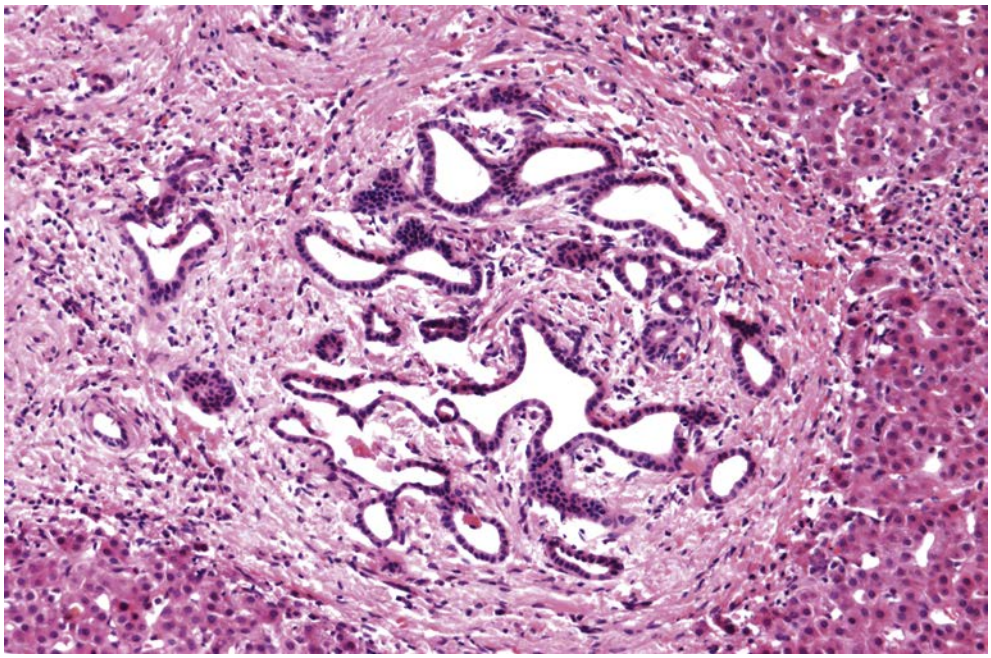

Fig. 13.11 Microhamartoma. A cluster of duct-like structures with irregular contours and focal dilatations is seen in a portal tract. Note resemblance to congenital hepatic fibrosis, shown in **Fig. 13.10**. (Wedge biopsy, H&E.)

Caroli's disease (congenital dilatation of the intrahepatic bile ducts)

This cystic malformation can affect different parts of the intrahepatic biliary tree and is seen alone or in combination with other congenital abnormalities, notably congenital hepatic fibrosis.[92] Because the cysts communicate with the rest of the biliary tree, there is a risk of ascending bacterial infection. Liver biopsy then shows the changes of cholangitis, with or without associated congenital hepatic fibrosis. The lesion of Caroli's disease must be distinguished from the acquired cholangiectases sometimes found in primary sclerosing cholangitis.[93]

Microhamartoma

Microhamartomas (von Meyenburg complexes, bile-duct malformations) are rounded nodules closely related to portal tracts, containing multiple biliary channels lined by regular epithelium and set in a stroma of dense fibrous tissue (**Fig. 13.11**). They may be grossly visible on the liver surface as white nodules 1–2 mm across. The lumens of the biliary structures sometimes contain inspissated bile. Serial sectioning shows that they are interconnected.[94] Microhamartomas are usually found incidentally and do not normally give rise to symptoms or abnormalities of liver function. They are often multiple, in which case they may very occasionally be associated with portal hypertension; distinction from congenital hepatic fibrosis is then difficult. Multiple lesions may be mistaken for metastatic tumour.[95] If a small nodule on the liver surface is seen during surgery, frozen section may occasionally be requested in order to exclude metastatic carcinoma. The irregularly dilated duct structures, inspissated bile and circumscription seen in microhamartomas are helpful in making this distinction.

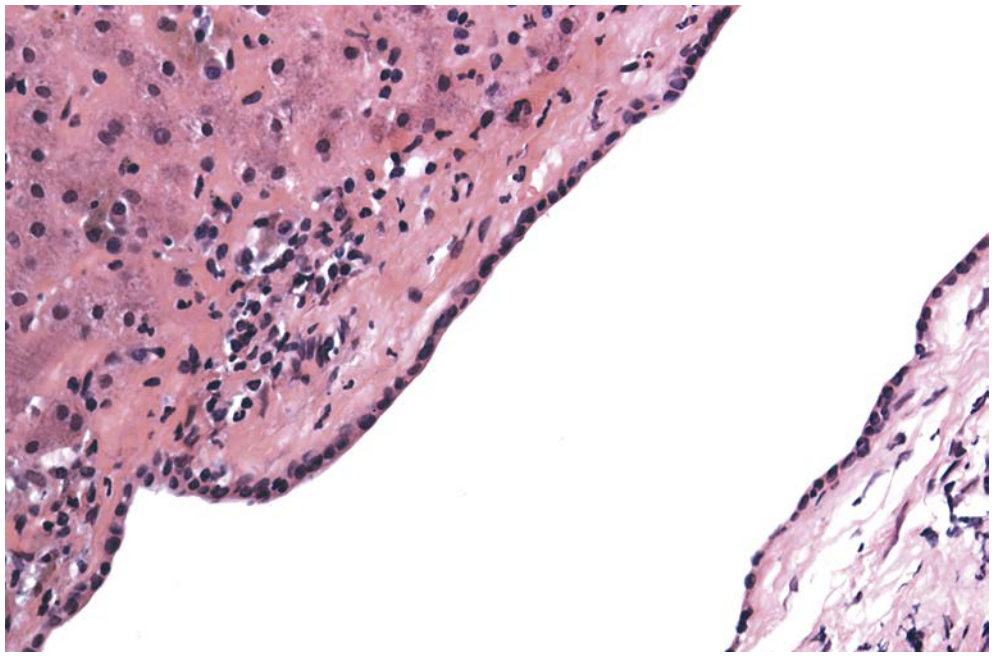

Fig. 13.12 Cystic liver. A cyst (top left) is lined by a single layer of low cuboidal epithelium. (Wedge biopsy, H&E.)

Polycystic disease

The infantile type of polycystic disease is regularly associated with renal involvement.[75,96] Portal tracts contain multiple cystic channels set in a fibrous stroma. In the adult type the cysts are lined by epithelium of biliary type (**Fig. 13.12**) but are not connected with the rest of the biliary tree. Solitary congenital cysts are histologically similar. The cuboidal or flattened epithelial lining helps distinguish these cysts from **ciliated hepatic foregut cysts** which are lined by ciliated cells and mucin-secreting goblet cells.[97] The presence of microhamartomas and features of Caroli's disease in individuals with polycystic disease favours a continuum in the expression of fibropolycystic disease.[98–100]

Inherited metabolic disorders

Cystic fibrosis

Cystic fibrosis (CF) is an inherited disease in which abnormally viscous exocrine secretions are present in the pancreas, salivary glands, alimentary tract and lungs. Liver disease is present in up to 10% of children but is very uncommon in adults with CF.[101–103] Jaundice in the neonatal period has been attributed to bile-duct obstruction by abnormally viscous bile and to gastrointestinal obstruction by meconium. Intercurrent hepatitis may also be responsible. Steatosis is common, although not always related to malnutrition.[104] Paucity of intrahepatic bile ducts in CF has also been reported.[105] In a proportion of older children a characteristic lesion of intrahepatic bile ducts is found.[106] Dense plugs of PAS-positive material are seen within dilated, proliferated ducts (**Fig. 13.13**). Bile-duct cells may undergo degeneration and necrosis.[104] There is surrounding fibrosis[107] and a variable degree of inflammatory infiltration which may be associated with abnormal intrahepatic ducts on cholangiography.[108] Eventually the fibrous areas may join, separating parenchymal islands. The term **focal biliary fibrosis** expresses the uneven involvement of the intrahepatic bile ducts in this process, with parts of the liver remaining unaffected. In

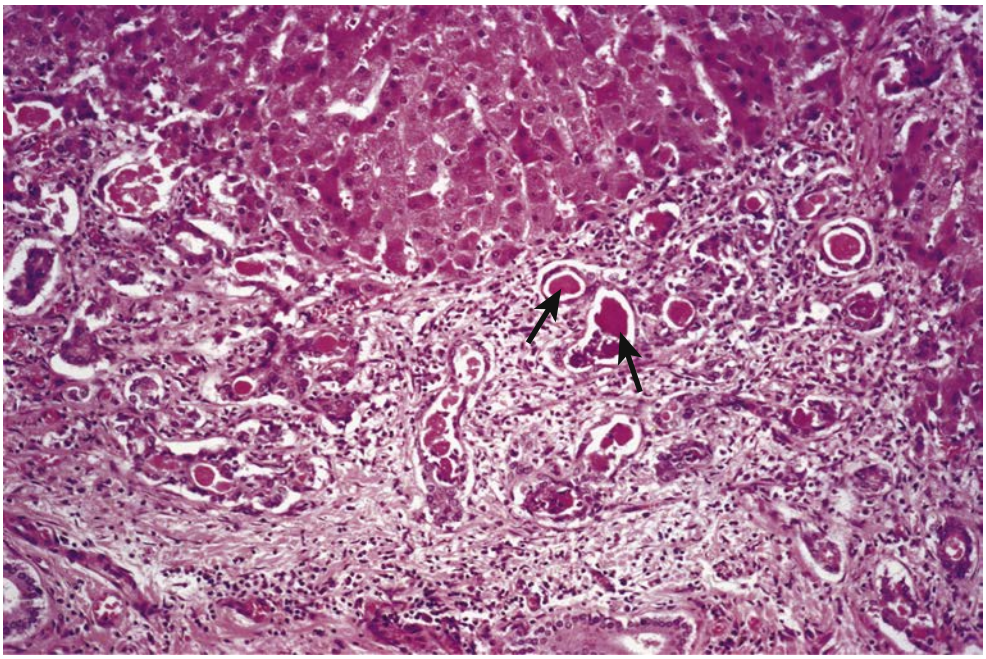

Fig. 13.13 Cystic fibrosis. Proliferated bile ducts in an enlarged, fibrosed portal tract contain dense inspissated material (arrows).

some patients the disease evolves to secondary biliary cirrhosis.[106] Adults with CF less often have the focal biliary fibrosis seen in children, but they may have steatosis due to alcohol consumption and/or risk factors for non-alcoholic fatty liver disease. Additional problems in adults include hepatolithiasis with secondary sclerosing cholangitis (due to increased lithogenicity of CF bile) and portal hypertension which may be due to biliary cirrhosis or may be non-cirrhotic portal hypertension (with evidence of obliterative portal venopathy or of nodular regenerative hyperplasia).[109]

Storage disorders: general remarks

Inherited metabolic defects leading to the abnormal accumulation of lipids, proteins and carbohydrates in the liver are many and varied; for a full description of the morphological changes, reviews should be consulted.[110–112] Ishak[110] helpfully discusses the differential diagnosis of individual histological features. Liver biopsy is sometimes useful in diagnosis, though by no means always decisive. The following points are offered as practical suggestions for occasions when biopsy is contemplated in children suspected of having storage disorders.

1. Storage disorders can involve hepatocytes (e.g. glycogenoses, α_1-antitrypsin deficiency), macrophages (e.g. Gaucher's disease) or both (e.g. Niemann–Pick disease, mucopolysaccharidoses,[113] cholesterol ester storage disease). When Kupffer cells are involved, they may swell to the size of hepatocytes and their involvement may not at first be apparent; the use of stains other than H&E, especially PAS and trichrome stains, then usually makes the Kupffer-cell involvement obvious.
2. Suspicion of a possible storage disorder is one of the few indications for electron microscopy of part of the biopsy specimen as a diagnostic procedure, because characteristic ultrastructural appearances sometimes enable a correct diagnosis to be established quickly.[114] Even when the changes are not diagnostic, they can direct attention to a particular group of diseases, and suggest the next line of investigation. Arrangements for electron microscopy should be made beforehand, so that

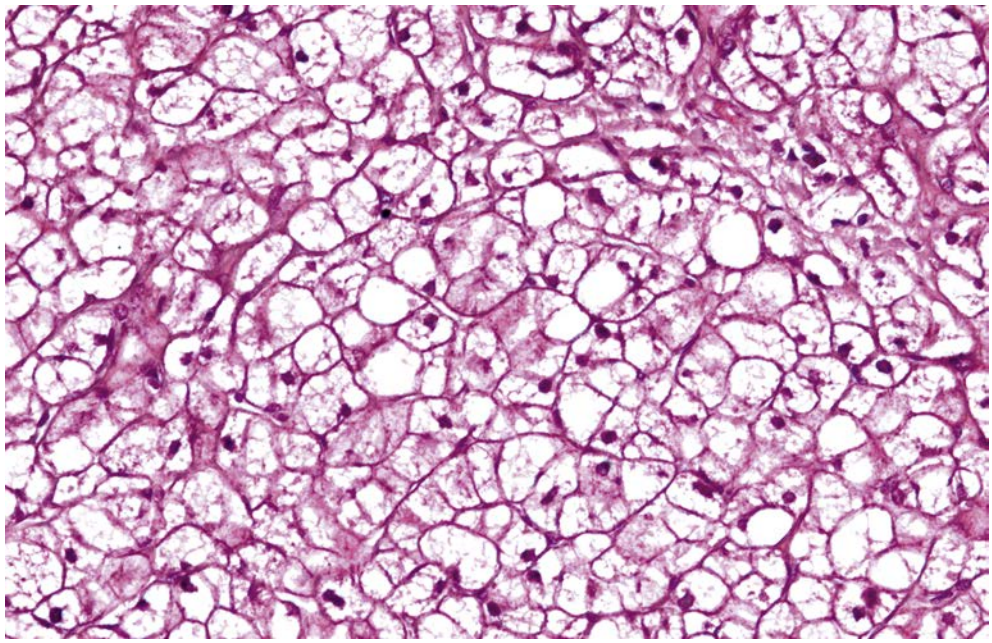

Fig. 13.14 Glycogenosis. In this example of type I glycogen storage disease, hepatocytes are swollen and resemble plant cells. (Wedge biopsy, H&E.)

part of the specimen can be put into the correct fixative without delay. In centres without facilities for electron microscopy, part of the specimen should still be correctly fixed and/or embedded, and sent to a referral centre later if light microscopic findings warrant this.

3. Arrangements should also be made to freeze part of the specimen and to store it in liquid nitrogen for possible biochemical analysis and histochemical staining of frozen sections. Speed is essential to avoid loss of enzyme activity. Again, a specialist centre may need to be consulted, because few centres or pathologists have the necessary expertise to investigate the rarer metabolic diseases.

Many inherited metabolic diseases affect the liver and several may lead to **cirrhosis**[110] (glycogenosis types III, IV and VI, galactosaemia, tyrosinaemia type I, α_1-antitrypsin deficiency, Wilson's disease, hereditary haemochromatosis). Liver transplantation may be indicated in some patients.[29,115,116] The discussion in this chapter will be limited primarily to the disorders mentioned under point 1 above. Haemochromatosis and Wilson's disease are described in **Chapter 14**.

Glycogen storage diseases (glycogenoses)

Most forms of glycogen storage disease involve the liver.[117,118] In type I glycogenosis (von Gierke's disease), fat and glycogen accumulate in the cytoplasm of hepatocytes. These appear swollen, pale-staining and sometimes vacuolated with H&E and have centrally placed nuclei (**Fig. 13.14**). Mallory–Denk bodies may be found in the cytoplasm.[119] The abundant glycogen displaces the organelles of affected cells to the periphery, giving them a plant-cell-like appearance. Some liver-cell nuclei also contain glycogen. Sinusoids are compressed. The overall appearance has been described as a uniform mosaic pattern.[117] Slender periportal fibrous scars often develop. Hepatocellular adenomas[120,121] or even, rarely, carcinomas[122] may develop. Rapid fixation in buffered formal saline usually enables abundant glycogen to be demonstrated in hepatocytes in paraffin sections, but it should

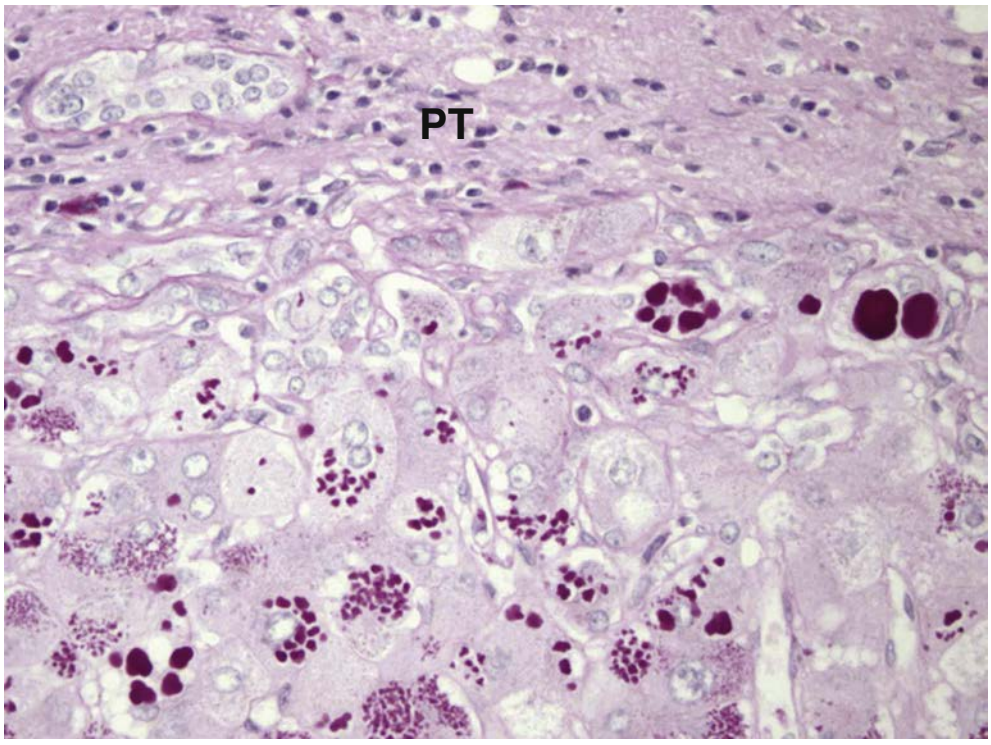

Fig. 13.15 Alpha$_1$-antitrypsin deficiency. Hepatocytes near a portal tract (PT) contain many magenta globules of different sizes. (Explant liver, diastase–PAS.)

be noted that the diagnosis does not rest only on the demonstration of glycogen, which is plentiful in normal liver. Features closely resembling type I glycogenosis can be seen in poorly controlled diabetics with **Mauriac syndrome** and **glycogenic hepatopathy** (Ch. 7).

In type II glycogenosis (Pompe's disease), the highly soluble storage material is contained in enlarged lysosomes, visible as vacuoles in hepatocytes and Kupffer cells by light microscopy. Many other tissues are involved. Type III glycogenosis has been subdivided into several biochemical subtypes. Histological appearances are like those of type I, but fat is less abundant and there may be fibrosis or cirrhosis.[123] Type IV (amylopectinosis) is characterised by abnormal glycogen in the form of well-defined cytoplasmic inclusions in hepatocytes[124] (Ch. 4). The glycogen is only incompletely removed by diastase digestion. The inclusions have a ground-glass appearance and must be distinguished from hepatitis B virus surface antigen and other similar cytoplasmic inclusions[125] (**see Fig. 4.4 and Table 4.1**). In other types of glycogenosis there is often much variation in the degree of hepatocellular swelling in different areas, in contrast to the regular distribution of the changes in type I.[117]

Alpha$_1$-antitrypsin deficiency

Individuals with decreased levels of the serum protease inhibitor α_1-antitrypsin (α_1-antitrypsin deficiency) may present with liver disease as neonates (neonatal cholestasis), in adolescence or in adulthood, even beyond 60 years of age.[126–128a] Recent data suggest that there are two peak ages at which severe α-1-antitrypsin (AAT) liver disease presents: birth to age 5, and ages 50–65.[128b] There are over 100 different alleles of the AAT gene,[129] two of which determine an individual's phenotype. The most common phenotype, PiMM, is associated with normal serum levels of AAT. Individuals with heterozygous (PiMZ) and homozygous (PiZZ) deficiency have moderately and profoundly reduced serum levels of AAT, respectively. The accumulation of characteristic PAS-positive, diastase-resistant globules in the hepatocytes of AAT-deficient individuals (Fig. 13.15) is based on a structural change in

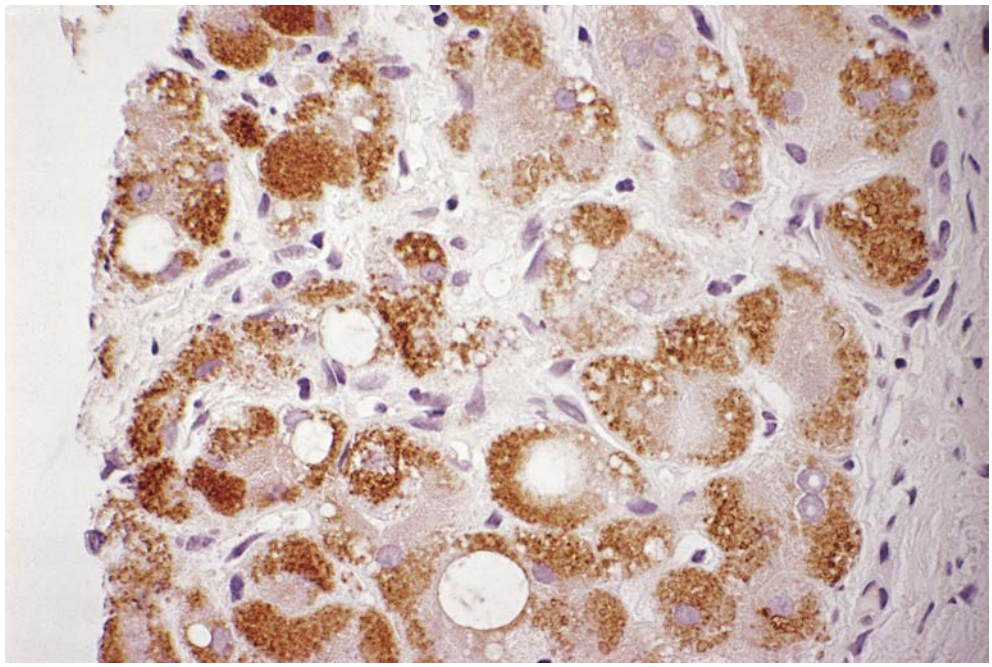

Fig. 13.16 Alpha$_1$-antitrypsin deficiency. Periportal hepatocytes contain numerous globules of α_1-antitrypsin, stained brown by the immunoperoxidase method. Typically, each globule is stained around the perimeter, with a central unstained region. (Needle biopsy, specific immunoperoxidase.)

the glycoprotein which is encoded by the mutant Z gene.[130] An amino acid substitution (lysine for glutamic acid at position 342) results in abnormal folding and polymerisation[131] of the protein and failure of both its secretion from the endoplasmic reticulum[130] and its subsequent degradation. In this regard AAT deficiency is conceptually similar to Alzheimer's and Parkinson's diseases, where inclusions result from conformational disorders of serine proteases (serpinopathies).[132]

The globules of AAT which accumulate range from less than 1 μm to 10 μm or more in diameter. They are mainly found in periportal hepatocytes, a similar distribution to the much smaller granules of copper-associated protein and haemosiderin, from which they need to be distinguished. In doubtful cases, immunohistochemical staining enables AAT to be identified with certainty (**Fig. 13.16**). Moreover, immunohistochemical staining is more sensitive than diastase–PAS positivity, and is helpful when diastase–PAS-positive globules are scanty or unevenly distributed. Conversely, immunohistochemically positive material is found in some patients without the genetic deficiency, usually with a panlobular or perivenular rather than a periportal distribution,[133,134] and particularly in livers with sinusoidal congestion and hypoxia.[135] From a practical point of view, it is wise to regard the presence of diastase-resistant PAS-positive globules in periportal liver cells as evidence for α_1-antitrypsin deficiency until proved otherwise.[136] Intracellular AAT globules have been vividly demonstrated in a transgenic mouse model of the disease.[137]

Some children with homozygous AAT deficiency develop neonatal cholestasis. Histological changes include a ductular reaction and fibrosis, but the typical globules may not be seen until the age of 3 or 4 months.[138] The subsequent course varies: many children improve, while others develop a chronic cholestatic syndrome with paucity of bile ducts or cirrhosis.[19,128a,139] Cirrhosis in children with AAT deficiency often has 'biliary' features, such as a ductular reaction and partial preservation of lobular architecture.

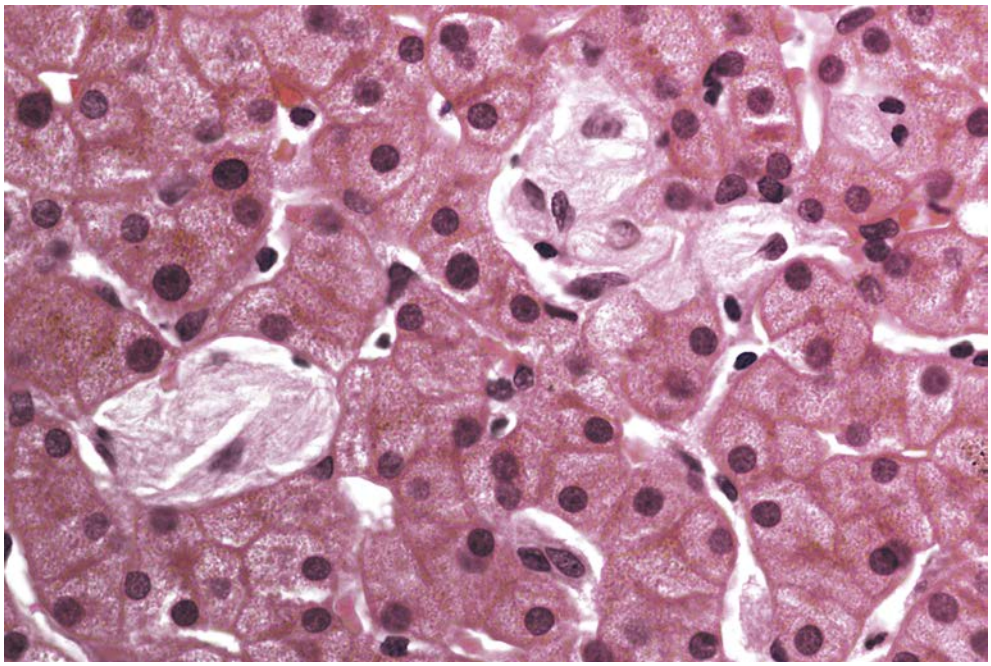

Fig. 13.17 Gaucher's disease. Pale-staining, striated Kupffer cells containing stored lipid are present within sinusoids. (Wedge biopsy, H&E.)

Adults carrying two Z alleles present with pulmonary emphysema or liver disease, but may also be symptom-free and healthy. Liver biopsy may show little apart from the PAS-positive globules or varying degrees of fibrosis. Cirrhosis develops in approximately one-third of homozygotes[129] and is either inactive or shows features of chronic hepatitis. An increased prevalence of hepatitis B and C viral infections in AAT deficiency may contribute to this picture.[140a] Steatosis and metabolic syndrome are often present and increase the risk of fibrosis.[140b] The characteristic globules are found predominantly in periportal or periseptal hepatocytes. They are seen most easily in sections stained with diastase–PAS, phosphotungstic acid–haematoxylin or specific immunoperoxidase, but are also seen in trichrome preparations and, when large and abundant, are faintly visible with H&E. Similar globules are seen in some hepatocellular carcinomas in patients with or without the Z allele.[141,142] Furthermore, an increased risk of hepatocellular carcinoma has been reported in male patients with AAT deficiency.[143] Chronic hepatitis, cirrhosis, large-cell liver-cell dysplasia and hepatocellular carcinoma may also be seen in heterozygous (PiMZ) AAT deficiency[144] and in individuals with other allelic variants such as Mmalton.[145–147] The prevailing concept regarding the pathogenesis of AAT-related liver disease is that while the primary insult is the accumulation of abnormal polymerised AAT within the endoplasmic reticulum, there are other *secondary* genetic, environmental and/or cellular 'hits' (e.g. activation of autophagy and endoplasmic reticulum stress pathways by enzyme retention).[148a] Epigenetic modifications, such as hypomethylation of certain genes associated with fibrosis, cancer and immune function, also affect the varied clinicopathologic presentations of AAT deficiency.[148b]

Brief mention should be made of several other endoplasmic reticulum inclusions found in hepatocytes in patients who may have chronic hepatitis or cirrhosis. Diastase–PAS-negative periportal granules of α_1-antichymotrypsin can be identified by specific immunohistochemical staining in **partial α_1-antichymotrypsin deficiency.**[149,150] In **fibrinogen storage disease** there are diastase–PAS-negative intracellular pale inclusions resembling ground-glass hepatocytes.[149,151]

Gaucher's disease (glycosyl ceramide lipidosis)

Cerebrosides accumulate in Kupffer cells and portal macrophages, which are enlarged, moderately diastase–PAS-positive and have a finely striated appearance (**Fig. 13.17**). The

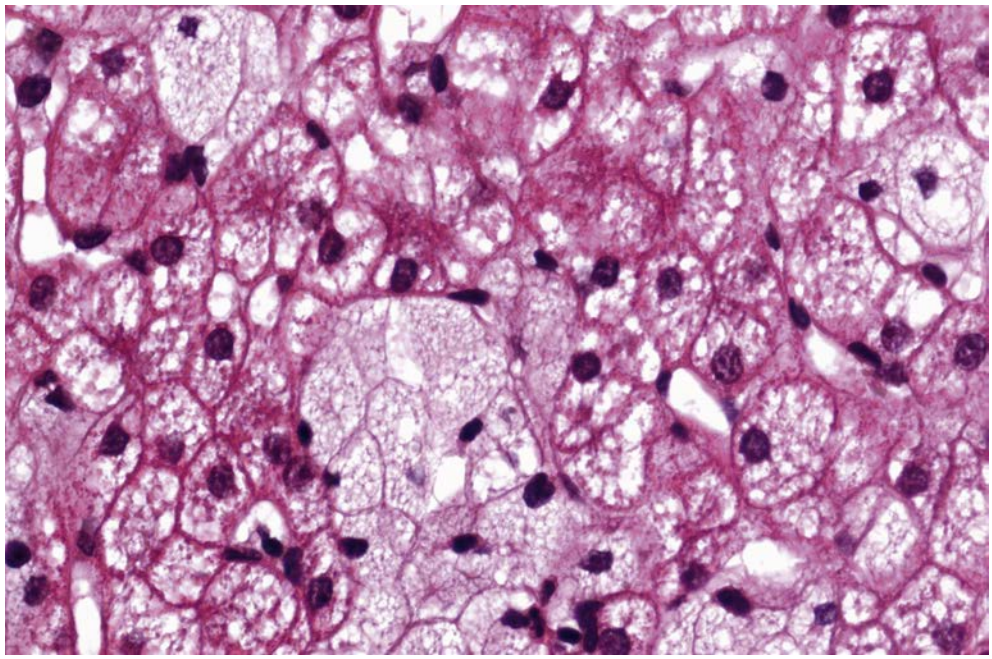

Fig. 13.18 Niemann–Pick disease. The darker cells are glycogen-rich periodic acid–Schiff-positive hepatocytes. Between them are large, pale-staining Kupffer cells filled with lipid. (Wedge biopsy, PAS.)

affected cells compress hepatocytes and sinusoids and may give rise to portal hypertension. Pericellular fibrosis is a common finding.[152]

Niemann–Pick disease (sphingomyelin lipidosis)

There are several variants of Niemann–Pick disease, and the clinical features range from severe and fatal neurological disease in infancy to symptomless hepatosplenomegaly in adults. The typical morphological feature of this disorder is the accumulation of sphingomyelin in both hepatocytes and macrophages. The latter are greatly swollen, foamy and diastase–PAS-positive to a variable extent. They can readily be distinguished from glycogen-rich liver cells in sections stained by the PAS method (**Fig. 13.18**). In addition to sphingomyelin, portal phagocytes, especially in the adult form, may also contain a brown lipofuscin-like pigment; these, as well as similar cells in bone marrow, stain a sea-blue colour by the Giemsa method. Niemann–Pick disease is thus one cause of the so-called sea-blue histiocyte syndrome.[153] Type B Niemann–Pick disease may progress to cirrhosis.[116,154]

Wolman's disease and cholesterol ester storage disease

In these apparently related conditions—the first a severe and usually fatal disease of infants, the second a milder disease of older children—cholesterol esters accumulate in hepatocytes and macrophages.[155,156] Hepatocytes also contain much triglyceride. The diagnosis may be suspected from the bright-orange colour of the liver biopsy core. By light microscopy, hepatocytes show microvesicular steatosis, and macrophages are enlarged and foamy.[157] Crystalline deposits may be seen within affected cells, particularly in frozen sections. The excess lipid is birefringent. Other features which may be found include ductular proliferation, pericellular fibrosis and even cirrhosis.[156]

Galactosaemia

Severe fatty change appears early in children with an inherited deficiency of galactose-1-phosphate uridyl transferase. Ductular reaction and cholestasis may also be present.

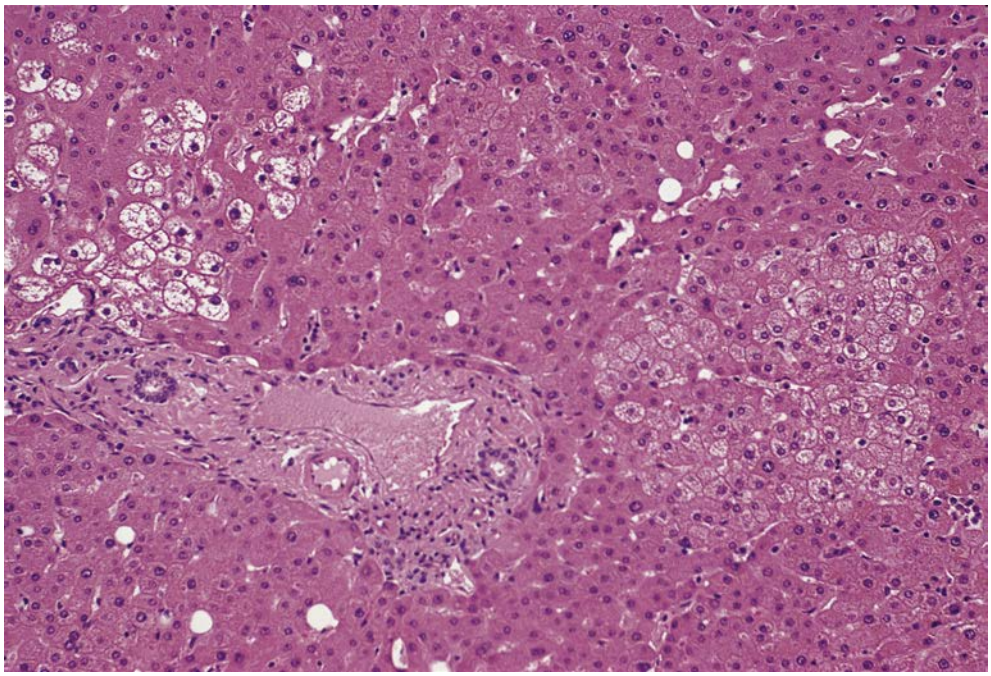

Fig. 13.19 Focal glycogenosis. Two foci of glycogen-containing hepatocytes with clear cytoplasm are seen near the portal tract. The patient had undergone partial hepatectomy for metastatic adenocarcinoma. In individuals with deficiencies of urea cycle enzymes, this lesion is accompanied by microvesicular steatosis. (Partial hepatectomy, H&E.)

Within a few weeks, liver-cell plates become transformed into tubular, duct-like structures (cholestatic rosettes) which dominate the histological picture, and there is siderosis and extramedullary haemopoiesis. Fibrosis and cirrhosis then develop. Institution of a galactose-free diet may result in substantial histological improvement.[158]

Histologically, the differential diagnosis includes **hereditary fructose intolerance**, in which the changes are somewhat similar but less severe. Also similar but more severe are the histological changes of **tyrosinaemia**. In this condition adenoma-like nodules are often seen, containing much fat.[110,111] Siderosis is also prominent. Hepatocellular carcinoma can develop, particularly in children over the age of 2 years, and liver transplantation is an important therapeutic option to forestall this event.[159]

Disorders of ureagenesis

Deficiencies in enzymes of the urea cycle, including ornithine transcarbamylase and carbamoyl phosphate synthase, may produce fatal hyperammonaemia in children and, rarely, in adults.[160] In these disorders the liver shows microvesicular steatosis which may be accompanied by aggregates of clear hepatocytes (**focal glycogenosis**[161]; **Fig. 13.19**). These glycogen-enriched regions stain brightly with PAS and are diagnostically helpful in excluding other causes of paediatric microvesicular fatty liver such as Reye's syndrome (see the next section).

Reye's syndrome

This is a serious and often fatal condition of encephalopathy and fatty change in the viscera of children under the age of 18 years. Viral infections (influenza B or A, varicella),

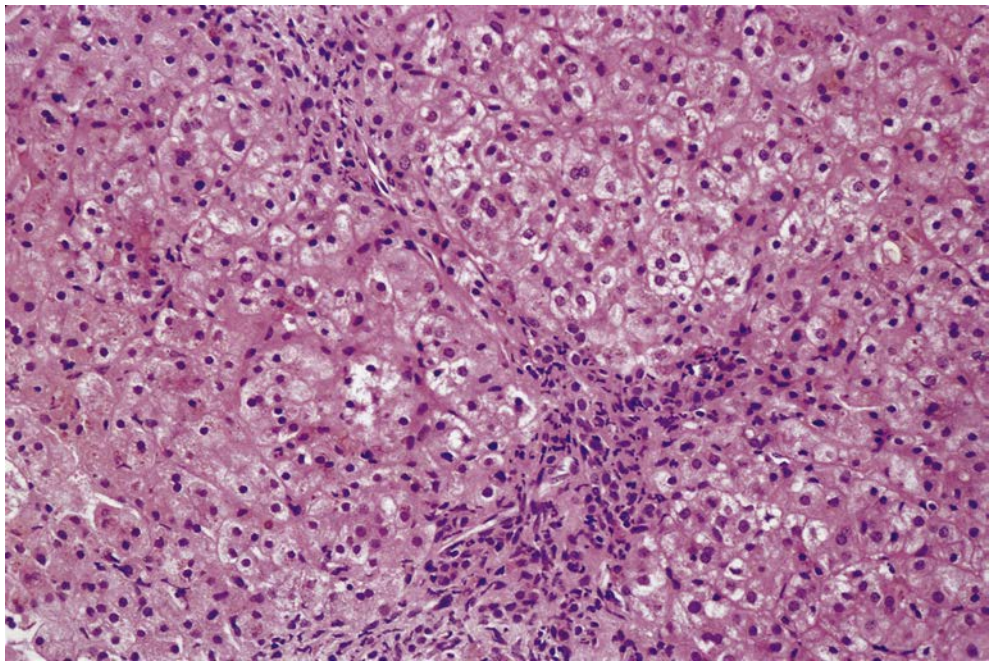

Fig. 13.20 Parenteral nutrition. An irregular, fibrotic portal tract shows proliferated bile ductules and a mixed inflammatory cell infiltrate of neutrophilic leukocytes and lymphocytes. Many cholestatic rosettes are present. (Needle biopsy, H&E.)

salicylate ingestion and endotoxaemia have been implicated in the pathogenesis.[162–164] The incidence of Reye's syndrome declined throughout the 1980s, parallel with a decrease in the use of salicylates for childhood viral illnesses. Rarely, it is still seen in some parts of the world.[165,166]

Liver biopsy is an important part of the investigation. The specimen is abnormally pale or yellow on naked-eye examination, and on light microscopy there is fine-droplet fatty change. This is panlobular in distribution and may be difficult to see without specific staining for fat, because of the small size of the vacuoles. Droplets are smaller in perivenular regions than elsewhere. Necrosis and inflammation are usually slight or absent, but in a few patients there is periportal ballooning or necrosis of hepatocytes.[167,168] Electron microscopy shows characteristic degenerative changes in liver-cell mitochondria; these are swollen and irregular in shape, with flocculent, electron-lucent matrix and reduced numbers of granules.[169] Succinic dehydrogenase activity is reduced. The differential diagnosis includes other conditions with microvesicular fat such as drug hepatotoxicity, urea cycle defects and mitochondrial hepatopathies associated with respiratory chain[170] and fatty acid oxidation defects.[4]

Parenteral nutritions

The effects of parenteral nutrition have been briefly mentioned in **Chapters 7 and 8**. It is pertinent to note here that in infants **cholestasis** is the major lesion associated with parenteral nutrition[171,172]; this may occasion diagnostic difficulties when other causes of cholestasis such as sepsis or biliary obstruction are also under clinical consideration. These difficulties are compounded by the fact that with prolonged administration of parenteral nutrition the portal tracts show progressive changes which are very similar to those of bile-duct obstruction and biliary atresia (**Fig. 13.20**). A ductular reaction may be present after 3 weeks of parenteral nutrition,[173] followed by portal fibrosis at 8–12 weeks and cirrhosis after 12 weeks.[174] Correlation of biopsy features with detailed clinical information regarding the duration of parenteral nutrition is clearly paramount in establishing the cause of

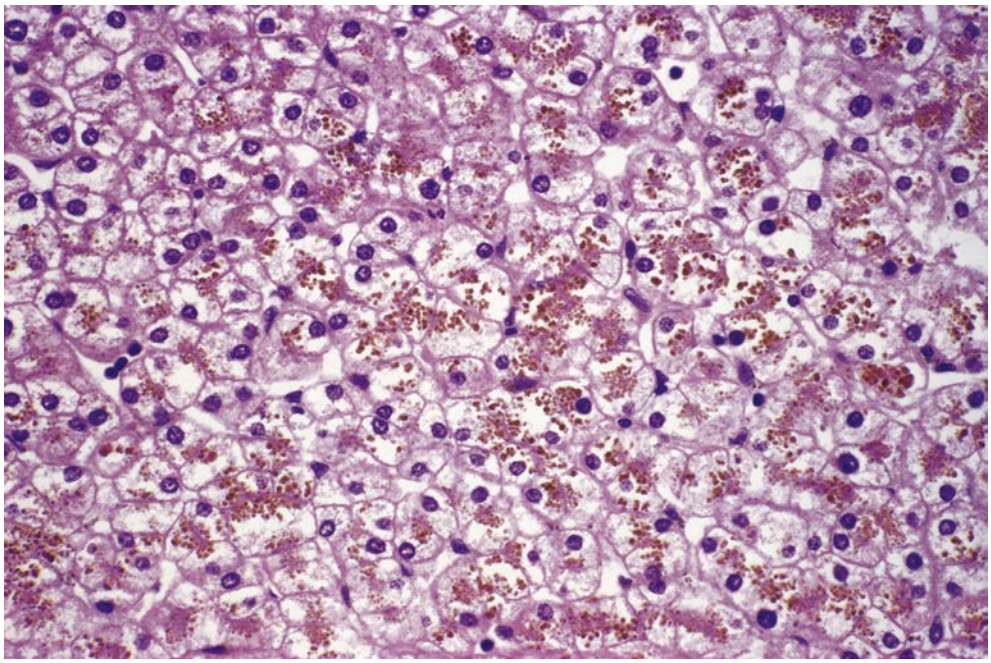

Fig. 13.21 Dubin–Johnson syndrome. Hepatocytes contain abundant coarse, dark-brown pigment granules. (Needle biopsy, H&E.)

jaundice in this population. Steatosis is less common in infants than in older children and adults who receive parenteral nutrition.[175] Even after parenteral nutrition is discontinued, steatosis as well as portal fibrosis may persist.[176]

Hyperbilirubinaemias

In **Gilbert's syndrome**, a common form of familial unconjugated hyperbilirubinaemia (the most common hereditary hyperbilirubinaemia affecting approximately 5%–10% of Caucasians),[177–179] the liver is histologically normal by light microscopy except for increased hepatocellular lipofuscin. In the **Dubin–Johnson syndrome**, in which the serum bilirubin is mainly conjugated, canalicular excretion of bilirubin and some other organic substances is defective[180] because of a mutation in the gene for canalicular multispecific-organic-anion transporter.[178,181] Other constituents of bile are excreted normally and there is no cholestasis. A complex dark brown pigment accumulates in hepatocytes, especially in perivenular areas, giving the liver a dark, speckled appearance to the naked eye (**Fig. 13.21**). The pigment granules somewhat resemble normal lipofuscin pigment and occupy a similar pericanalicular site in hepatocytes, but are darker, more abundant, larger and more variable in size (**Fig. 13.21**). When the pigment is very abundant, its pericanalicular location is no longer evident. Simple histochemical characteristics such as PAS-positivity and acid-fastness do not reliably distinguish between Dubin–Johnson pigment and lipofuscin because both stain variably (**see Table 3.1**), but the distinction is usually clear on the basis of the aforementioned morphological features. When there is doubt, this may be resolved by electron microscopy, which shows the Dubin–Johnson pigment granules to be composed of characteristic strands of electron-dense material in an electron-lucent background, together with scanty lipid droplets (**see Fig. 17.2**).

Inherited cholestatic syndromes

Consideration of this group of disorders should be prompted when bland canalicular or canalicular and hepatocellular cholestasis with or without giant-cell transformation

are the predominant histological features. This picture may develop owing to a variety of mutations of bile transport proteins or inborn errors of bile acid synthesis. The morphological assessment should take into account not only the presence or absence of giant cells, but also whether native bile ducts are injured or absent and whether a mild ductular reaction and/or portal/periportal fibrosis or cirrhosis are present, because such features may help to distinguish among aetiologies.[1] Electron microscopy to assess the appearance of the bile and immunohistochemical staining to evaluate preservation or absence of specific bile transport proteins (e.g. bile-salt export pump (BSEP),[182] multidrug resistance protein 3 (MDR3)[183]) and others provide additional diagnostic information.

The cholestatic group of diseases termed **progressive familial intrahepatic cholestasis (PFIC)** has recently expanded beyond the original well-characterised three types so that currently there are five designated types of PFIC (types 1–5) with distinctive gene mutations, histopathological changes and, except for PFIC type 3, low to normal serum γ-glutamyl transferase (GGT) level[184a,184b,184c] (**Table 13.2**). These are autosomal recessive disorders in which gene mutations result in defective bile-salt transporter proteins on the canalicular membrane[181,183–199] (**Table 13.2**). Affected infants have jaundice, pruritus and intrahepatic cholestasis which may progress to cirrhosis early, or in later childhood. An unusually low or normal serum GGT level should raise the possibility of PFIC type 1 or 2 (**Table 13.2**). The best known of these is **PFIC-1 (Byler disease)**, which was originally described in kindred of the Amish settler Jacob Byler.[195] PFIC-1 is caused by mutations in *ATP8B1* (chromosome 18q21–q22) that encode FIC-1 protein, which is expressed on bile canaliculi and intestinal epithelium. In **PFIC-2 (Byler syndrome)**, there are mutations in *ABCB11* (chromosome 2q24.3–2q31.1) which encode BSEP, which is selectively expressed on bile canaliculi. The presence of normal to low levels of serum GGT relative to the degree of cholestasis is an important diagnostic feature of both PFIC types 1 and 2. The bland bile canalicular cholestasis of PFIC-1 (**Fig. 13.22**) contrasts with the features of 'neonatal hepatitis' (giant cells, inflammation, lobular disturbance) and progressive periportal fibrosis seen in PFIC-2 (**Fig. 13.23**). Electron microscopy shows distinctive coarsely granular bile in PFIC-1 (Byler disease) and filamentous or amorphous bile in PFIC-2 (BSEP deficiency; Byler syndrome)[195] (**see Fig. 17.11**). Portal fibrosis, ductular reaction and cirrhosis eventually develop in PFIC-2 (**Fig. 13.24**), sometimes in very young infants less than a year of age.[186] Hepatocellular carcinoma[182,200] and cholangiocarcinoma[201] are other reported sequelae. Many PFIC-1 patients develop graft steatosis after liver transplantation, possibly because of the continued expression of dysfunctional FIC-1 protein on intestinal epithelium.[202,203] Steatohepatitis with cirrhosis are additional complications.[204] **PFIC-3** is associated with mutations in the *ABCB4* gene (chromosome 7q21.1) which encodes MDR3. Biopsy shows bile canalicular cholestasis (occasionally with hepatocellular and/or ductular cholestasis), portal fibrosis and prominent ductular reaction and progression to a biliary-type cirrhosis (**Fig. 13.24**).[186] The most recently added PFIC types are **PFIC-4** (with protein-truncating mutations in the gene *TJP2*—tight junction protein 2—resulting in diminished claudin-1 expression at the bile canalicular tight junction[187,188]), **PFIC-5** (with mutations in the *FXR* gene—farnesoid X receptor—a nuclear receptor critical to bile-acid synthesis and homeostasis, and with secondary effects on expression of BSEP on bile canalicular membranes[189,190]) and, nominally, **PFIC-6** (*MYO5B* gene mutation and myosin 5b deficits resulting in defective cycling of microvillus elements to the apices of bile canalicular membranes and intestinal enterocytes, with or without associated intestinal microvillous inclusion disease (MVID)[192,193,197a]). Canalicular immunostaining for both BSEP and MDR3 is granular, thick and often overruns the canalicular membrane borders (**Fig. 13.25**). A recent additional familial neonatal cholestatic disorder (a putative PFIC type 7 with mutations of the *PLEC* gene) affects the cytoskeleton linker protein plectin that controls cytoskeletal keratin 8 intermediate filament binding to junctional complexes.[197b,197c] It should be noted that phenotypic variations among PFIC cases may cause loss of function and cholestasis, but genetic variants among the bile salt transporter genes can result in paradoxically

Table 13.2 Histological features of progressive familial intrahepatic cholestasis (PFIC).

Disorder (Synonyms) Gene/protein	Liver histopathology (*Serum GGT level*)
PFIC-1 (Byler disease) (FIC-1 deficiency) *ATB8B1*/FIC-1	Bland canalicular cholestasis Giant-cell transformation uncommon Little or no ductular reaction Occasional paucity of intrahepatic bile ducts (late) Slower progression than PFIC-2 Coarse bile (Byler bile) on electron microscopy (***Low or normal GGT***)
PFIC-2 (Byler syndrome) *(BSEP deficiency)* *ABCB11*/BSEP	Bile canalicular and hepatocellular cholestasis (zone 3 > zone 1) Giant-cell transformation common Greater lobular disturbance than PFIC-1 Perivenular, pericellular and periportal fibrosis with progression to cirrhosis (sometimes <1 year of age) Mild ductular reaction (later) Occasional interlobular bile-duct paucity (later) Hepatocellular carcinoma and cholangiocarcinoma have been reported Recurrent cholestasis after liver transplant in some who develop IgG anti-BSEP antibodies Negative immunostain for BSEP Hepatocellular carcinomas and cholangiocarcinomas may develop as sequelae (***Low or normal GGT***)
PFIC-3 *(MDR3 deficiency)* *ABCB4*/MDR3	Hepatocellular cholestasis with occasional canalicular and ductular cholestasis Ductular reaction prominent (resembles biliary obstruction) Portal/periportal fibrosis with cirrhosis Negative immunostain for MDR3 (***High GGT***)
PFIC-4 (*TJP2)*TJP2	Canalicular cholestasis Portal/lobular fibrosis Giant-cell transformation Hepatocyte necrosis Absent or reduced staining for claudin-1 on bile canaliculi (***Low to normal GGT***)
PFIC-5 (*NR1H4*) FXR	Canalicular cholestasis Diffuse giant-cell transformation Ductular reaction Absent FXR staining on bile canaliculus Absent BSEP staining on bile canaliculus (***Low to normal GGT***)
Other cholestatic disorder* (? 'PFIC-6') (*MYO5B*)/Myosin 5b (with or without intestinal MID)	Canalicular cholestasis Sparse giant-cell transformation Abnormally thickened and granular BSEP and MDR3 bile canalicular staining with overflow subcanalicular staining Mild periportal fibrosis (**Low to normal GGT**)

*Not officially designated as PFIC-6, but potential candidate cholestatic disorder.

BSEP, Bile-salt export pump; FXR, farnesoid X receptor; GGT, γ-glutamyl transferase; MDR3, multidrug resistance protein 3; MID, microvillus inclusion disease; TJP2, tight junction protein 2.

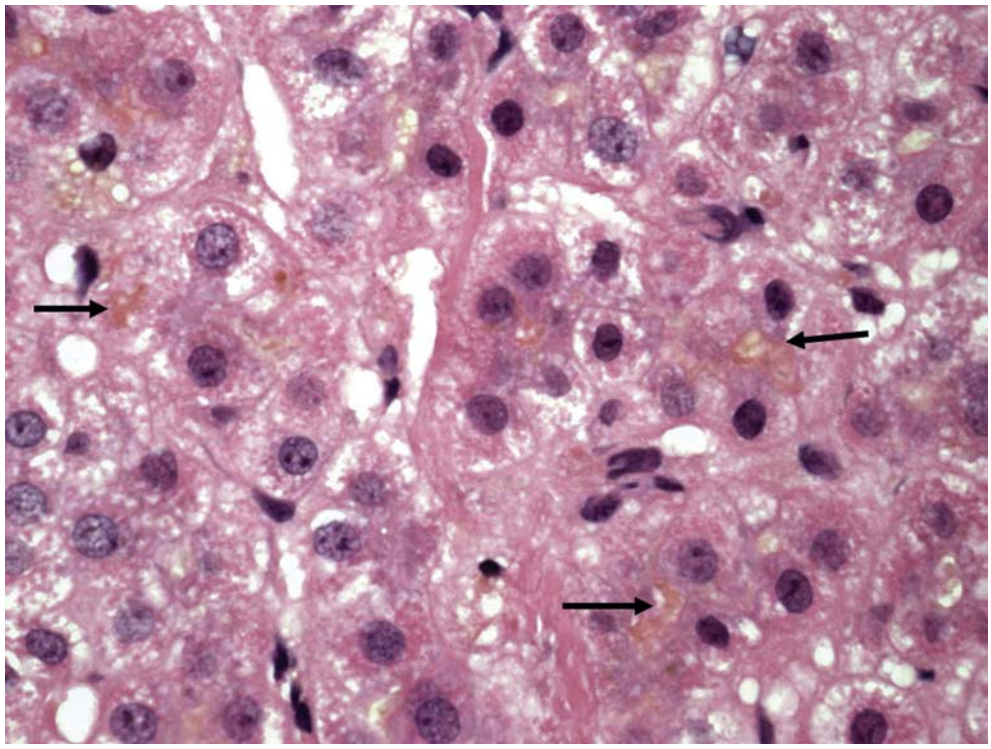

Fig. 13.22 Progressive familial intrahepatic cholestasis type 1 (PFIC-1) (Byler disease). Bland canalicular bile (arrows) is present, with relatively unperturbed parenchyma. (Needle biopsy, H&E.)

intact immunohistochemical expression of one or several affected bile-salt transporter proteins; in these circumstances, genomic analysis should be pursued in order to clarify the type of transporter disorder.[194a,194b,194c]

Episodic cholestasis is seen in two subtypes of **benign recurrent intrahepatic cholestasis (BRIC)**.[205] Subtype 1 shows a gene mutation mapped to *ATP8B1* on chromosome 18 (as in PFIC-1), and subtype 2 has a mutation in the *ABCB11* gene (also the target in PFIC-2).[206] Affected patients have multiple attacks of jaundice and itching, often starting in childhood or early adult life[181,183,207] and often triggered by a minor viral infection. Histologically, canalicular cholestasis is seen in attacks, usually unaccompanied by any substantial degree of inflammation (**Fig. 13.26**). Between attacks the liver returns to normal and there is no fibrosis or progression to cirrhosis. A clinical continuum between BRIC and PFIC is suggested in some cases.[208]

Additional congenital or familial cholestatic syndromes are described,[209] including Norwegian cholestasis, North American Indian cholestasis,[54,196] Navajo neurohepatopathy[3] and recurrent cholestasis in the Faeroe Islands.[210] Some children with MVID of the intestine also develop jaundice, pruritus and bile canalicular cholestasis with abnormally accentuated canalicular and cytoplasmic BSEP expression on immunostain as a result of inherent deficits in endosomal trafficking to epithelial cell membranes.[191,211]

Cirrhosis in childhood

Children are susceptible to many of the causal cirrhotic agents affecting adults, including hepatitis virus infections. As already noted, several inherited metabolic disorders lead to cirrhosis, and the possibility of Wilson's disease should always be considered in a child with chronic liver disease. Cirrhosis in young women should raise the question

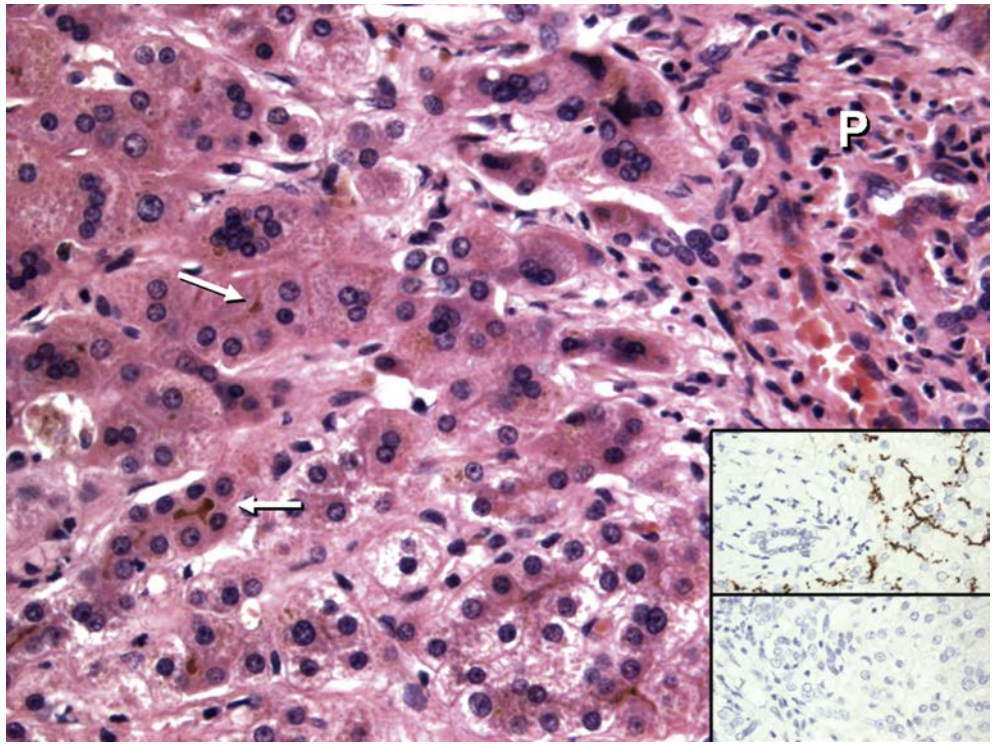

Fig. 13.23 Progressive familial intrahepatic cholestasis type 2 (PFIC-2) (bile-salt export pump (BSEP) deficiency). Cholestasis is present within bile canaliculi (arrows) and hepatocytes, accompanied by numerous multinucleated giant hepatocytes. The portal tract (P) shown is inflamed and fibrotic. Elsewhere in the specimen ductular reaction and developing cirrhosis were seen. (Explant liver, H&E.) Inset: Immunostain for BSEP shows strong bile canalicular positivity in the control (top), but absent staining in this specimen (bottom). (Explant liver, specific immunohistochemistry.)

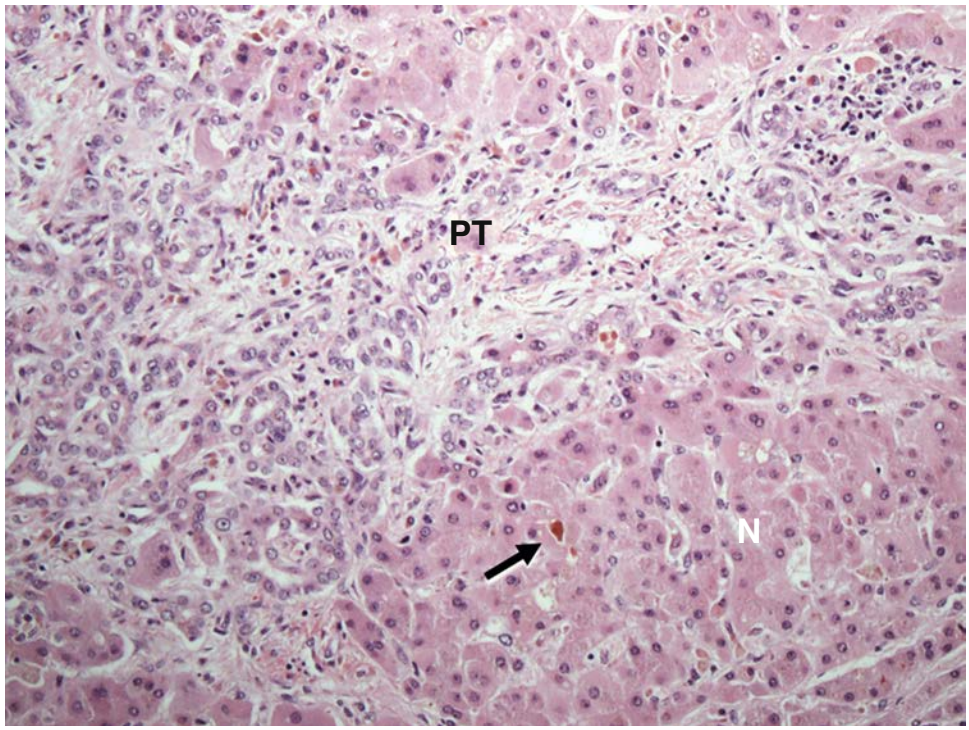

Fig. 13.24 Progressive familial intrahepatic cholestasis type 3 (MDR3 deficiency). Developing biliary cirrhosis is present in this case of PFIC type 3, with regenerative nodules (N) and prominent bile plugs within canaliculi (arrow). The portal tract (PT) at centre is expanded by fibrosis accompanied by a robust ductular reaction. (Explant liver, H&E).

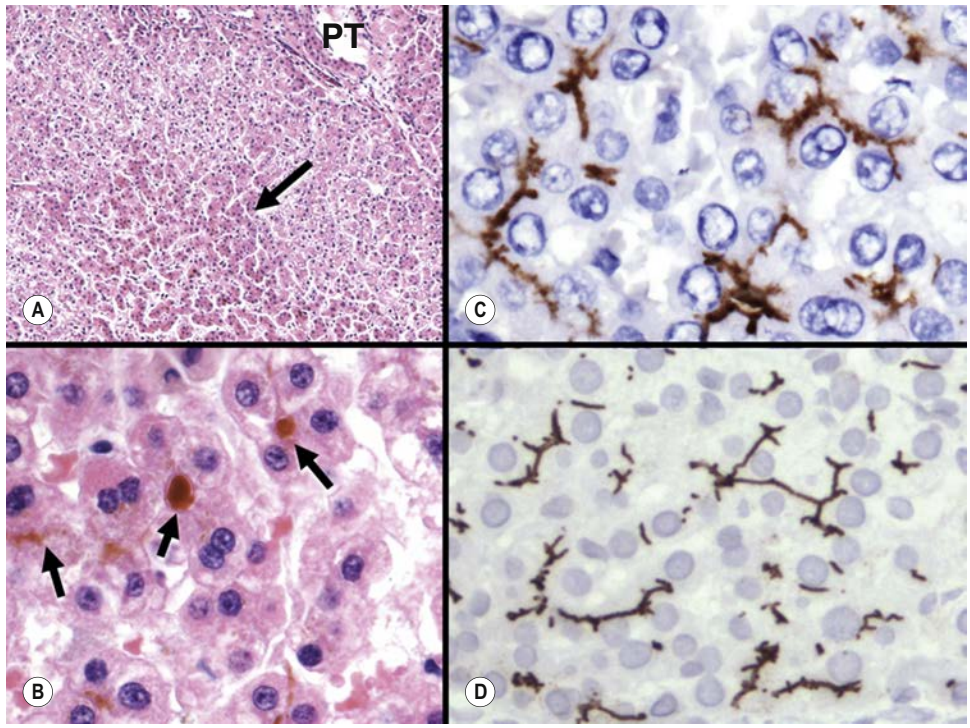

Fig. 13.25 Progressive familial intrahepatic cholestasis (PFIC) type 6 (MYO5B mutation in microvillous inclusion disease). A: The centrilobular regions (arrow) show prominent bile canalicular cholestasis with zonal prominence of cholestatic liver-cell rosettes. **B:** Canalicular bile plugs are prominent. **C:** Bile-salt export pump (BSEP) immunostain results are distinctly abnormal, with thick, irregular and granular positivity that overextends into the canalicular membrane. **D:** Control (non-PFIC) liver shows delicate branching network of uniformly thin and regular bile canaliculi with BSEP immunostain (Explant liver; **A** and **B:** H&E; **C** and **D:** specific immunoperoxidase).

of AIH, either type I (with anti-actin antibodies) or type II (anti-liver–kidney microsomal antibodies)[212] (Ch. 9). Rare familial forms of cirrhosis have been described.[213] Not infrequently, the aetiology of some forms of childhood cirrhosis is obscure, as for example in the cerebral degenerative disorder Alpers' disease,[214] in which microvesicular fat is also present. Cryptogenic cirrhosis due to keratin mutations[215] is another consideration (Ch. 10).

Indian childhood cirrhosis, a disease of high mortality affecting young Indian children (and occasionally reported from outside the Indian subcontinent[216–222]), greatly declined in incidence after the mid-1990s, when brass- and copper-containing vessels used for milk feeding were identified as sources of copper contamination.[223,224] The major features include hepatocellular swelling at an early stage followed by ballooning, Mallory–Denk body formation and necrosis. Focal accumulations of neutrophils and pericellular fibrosis resemble steatohepatitis, but there is little or no fatty change (Fig. 13.27). Large amounts of copper and copper-associated protein accumulate in affected hepatocytes,[225,226] and chelation therapy with D-penicillamine therapy is effective in some patients.[227] The small clusters of damaged hepatocytes surrounded by fibrosis eventually evolve to a cirrhosis characterised by very small nodules (micro-micronodular cirrhosis).

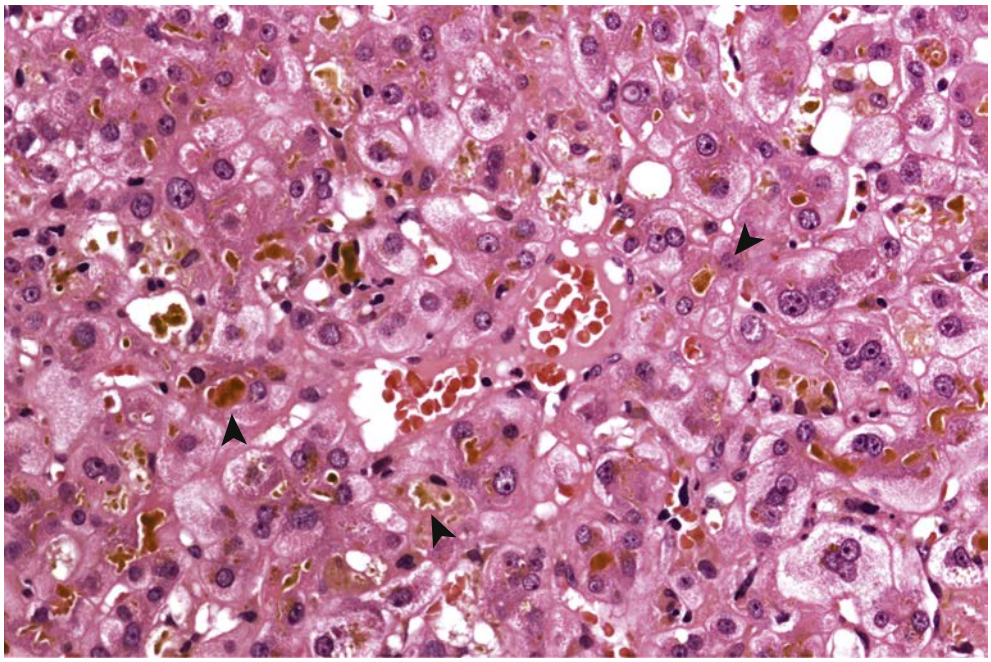

Fig. 13.26 Benign recurrent intrahepatic cholestasis. Bile canalicular cholestasis is diffusely prominent (arrowheads). (Needle biopsy, H&E.)

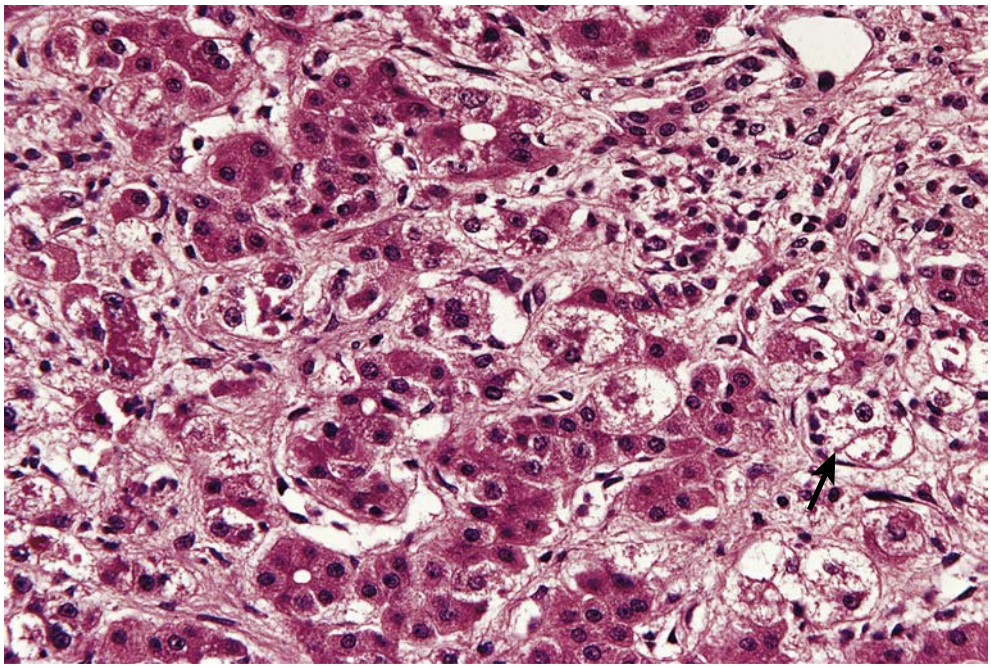

Fig. 13.27 Indian childhood cirrhosis. Many liver cells are swollen (centre) and surrounded by fibrosis and mononuclear cells. Mallory–Denk bodies are present within some hepatocytes (arrow). Regenerating hepatocytes are organised into small clusters. (Postmortem liver, H&E.)

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CHAPTER 14

Disturbances of Copper and Iron Metabolism

Wilson's disease (hepatolenticular degeneration)

Wilson's disease is an autosomal recessive disorder due to mutations in the gene *ATP7B* for copper-transporting ATPase located in the *trans*-Golgi network of the liver.[1] It is uncommon but important and treatable. Normal hepatic copper transport[2] is disrupted owing to various ATP7B mutations,[3] leading to the accumulation of copper in hepatocytes and liver disease. The large number and diverse mutations identified currently preclude simple genetic testing,[4] in contrast to hereditary haemochromatosis (discussed later). Liver biopsy is important for histological diagnosis and monitoring.[5]

Chemical quantitation of copper concentration in the biopsy sample helps to establish the diagnosis and is sometimes used for determination of the genetic status of a patient's siblings.[6,7] Copper determination can be made from specimens obtained by routine liver biopsy or retrieved from paraffin blocks, without special copper-free solutions or instruments.[8] Homozygous individuals have increased liver copper levels from an early age but do not develop symptoms of liver disease in the first few years of life. Increased liver copper levels precede the development of histological abnormalities. Hepatic copper levels are typically greater than 4 μmol/g dry weight (>250 μg/g dry weight).[8]

Histological lesions develop before the disease is clinically apparent. In the early, precirrhotic phase there is fatty change,[7] sometimes with the formation of fat granulomas.[6] Slender fibrous septa extend from portal tracts (**Fig. 14.1**). There may be unusually abundant lipofuscin pigment in hepatocytes and glycogen vacuolation of hepatocyte nuclei, but neither feature is easy to evaluate; both are found in normal individuals, and nuclear vacuolation is particularly common in the young. Lipofuscin granules may be larger and less regular in outline than normal,[9] possibly due to increased numbers of autophagic vacuoles which develop as protection against copper cytotoxicity.[10] Inflammation is absent or mild in the early stages. Kupffer cells are sometimes enlarged and may stain for iron as a result of haemolysis. Electron microscopy helps in the diagnosis of both early and late disease because of characteristic changes in mitochondria and lysosomes (**Ch. 17**).

In some patients a phase of chronic hepatitis develops next that is difficult to distinguish histologically from chronic viral hepatitis. Stains for copper and copper-associated protein may be helpful, as will be discussed later. Cirrhosis develops in untreated patients, with or without a recognisable preceding phase of chronic hepatitis. A common though not invariable pattern is of active cirrhosis with fatty change, ballooned hepatocytes, focally dense eosinophilic cytoplasm and glycogen vacuolation of nuclei (**Fig. 14.2**). Cholestasis may be present. Hepatocytes often contain Mallory–Denk bodies and these are sometimes very abundant. They are associated with an infiltrate rich in neutrophils, as in steatohepatitis (**Fig. 14.3**). Partial fibrous occlusion of efferent veins has been reported.[9] Hepatocellular carcinoma is a rare sequel of cirrhosis in Wilson's disease.[11,12]

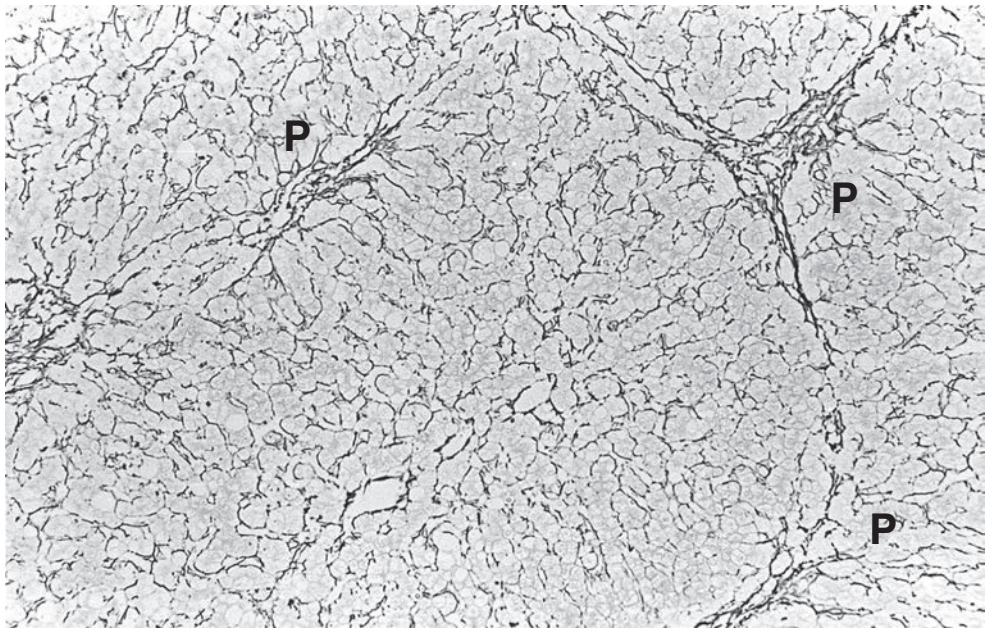

Fig. 14.1 Wilson's disease. At this early stage slender septa extend from portal tracts (P) but acinar architecture is intact. There is steatosis, just visible in this reticulin preparation. (Wedge biopsy, reticulin.)

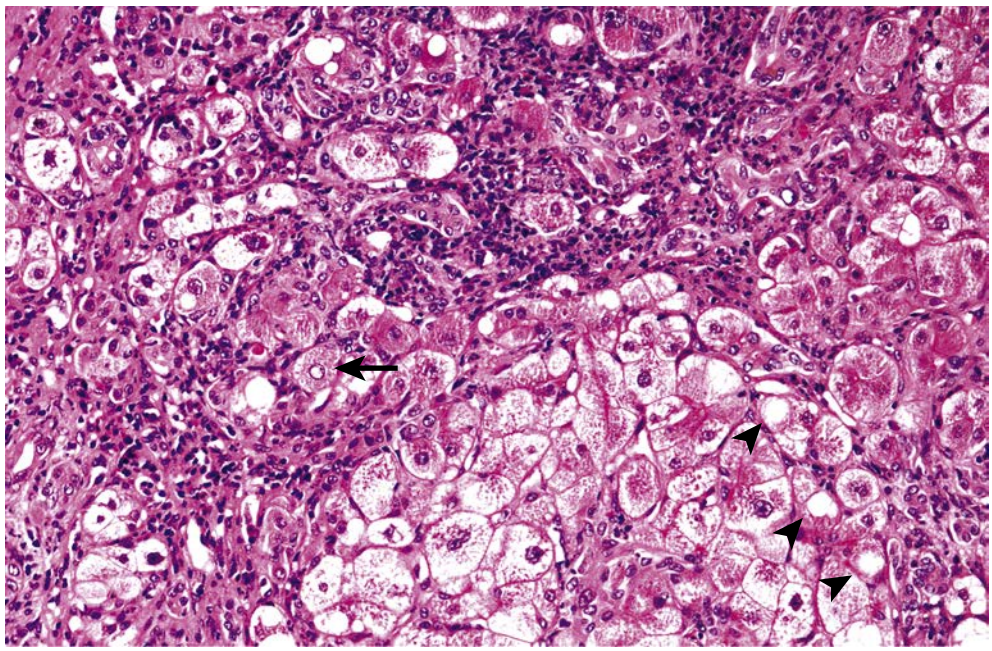

Fig. 14.2 Wilson's disease. Active cirrhosis with liver-cell swelling, steatosis (arrowheads) and nuclear vacuolation (arrow). (Wedge biopsy, H&E.)

Fulminant hepatic failure may be the first manifestation of Wilson's disease and is a major indication for liver transplantation.[13] The presence of haemolysis in a young individual with acute liver failure should therefore prompt consideration of Wilson's disease.[14] Cirrhosis is usually already present in such cases,[15,16] in contradistinction to acute liver failure, owing to viral or drug hepatitis where recent massive necrosis is evident. The cirrhotic

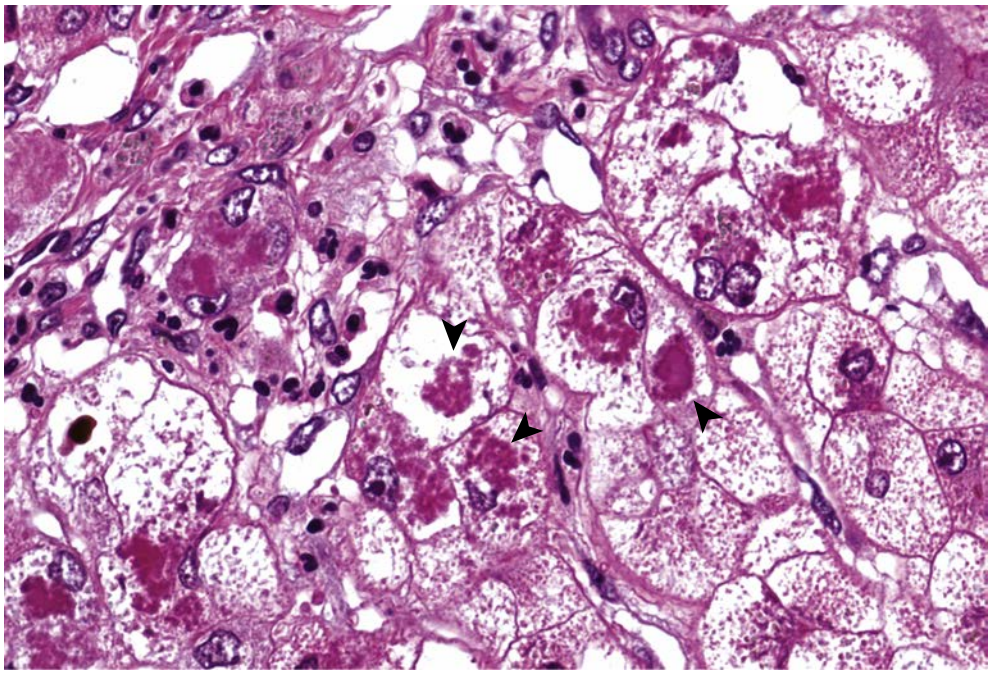

Fig. 14.3 Wilson's disease. Numerous Mallory–Denk bodies (arrowheads) are seen within hepatocytes. (Postmortem liver, H&E.)

nodules are frequently small and separated by septa containing abundant ductular structures and variable chronic inflammatory cells (**Fig. 14.4**). The death of hepatocytes in the fulminant disease occurs by both apoptosis and necrosis,[17] resulting in new zones of confluent necrosis superimposed on the underlying cirrhotic architecture. Cholestasis is often striking, and hepatocytes may contain large- or small-droplet fat. The presence of much stainable copper and/or copper-associated protein in hepatocytes and Kupffer cells distinguishes Wilson's disease from other causes of fulminant hepatic failure.

Staining for copper and copper-associated protein plays a part in the diagnosis of Wilson's disease, though staining results (as well as the copper concentration) can vary considerably throughout the liver.[18] Failure to stain in either case is common at some stages of the disease and does not therefore exclude the diagnosis. Conversely, both copper and copper-associated protein are found in other liver diseases, usually as a result of failure to secrete copper into the bile. Thus, in a child with liver disease, strong staining for copper might reflect loss of bile ducts rather than Wilson's disease. Other copper storage disorders have been described, including Indian childhood cirrhosis (**Ch. 13** and **Fig. 13.27**), which is also occasionally seen elsewhere in the world.[19–21] Furthermore, neonatal liver is normally rich in copper.[22a] Rarely, biopsies with substantial iron overload and haemosiderin deposits may also show positive copper staining, even in the absence of significant quantitative copper overload. This has been attributed to co-localization in lysosomes of several copper-containing proteins (cuproproteins) such as multicopper oxidases and copper/zinc superoxide dismutase that are involved in control of the redox state.[22b]

In the early phases of Wilson's disease, liver copper levels are high, but the copper is difficult to demonstrate histochemically. This is because it is diffusely distributed in hepatocytes and not concentrated in lysosomes. Sensitive histochemical methods (e.g. Timm's silver method or rhodanine) may show faint cytoplasmic staining. Later in the course of the disease copper begins to accumulate in hepatocyte lysosomes and is then more easily stained. Once cirrhosis has developed, the distribution of copper is typically uneven, some nodules staining strongly while others are negative (**Fig. 14.5**). Staining for copper and copper–protein may be dissociated, although in most cases both are positive.[23,24] Timm's silver stain appears to be the most sensitive staining method for demonstrating copper in this disease.[25]

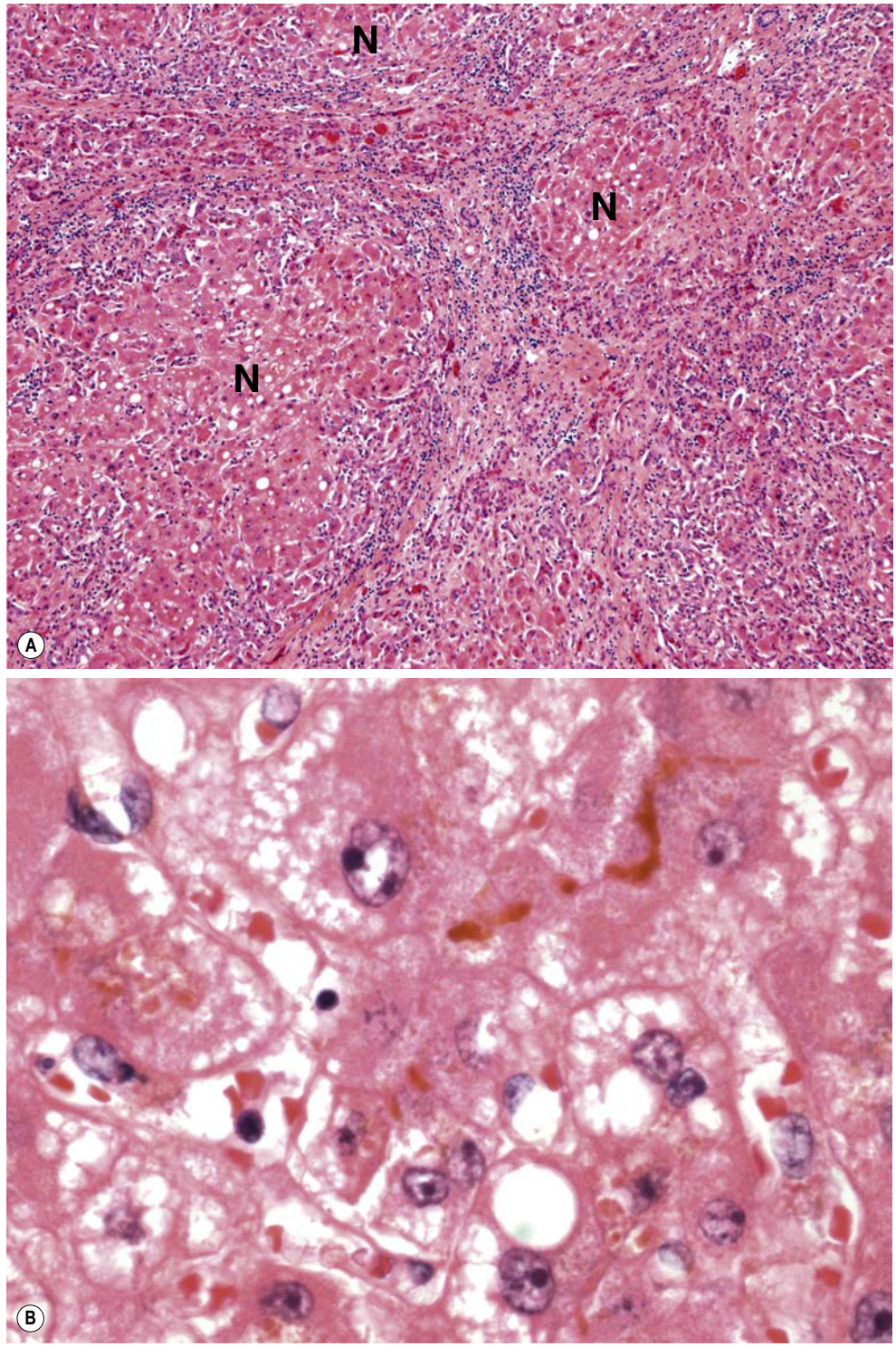

Fig. 14.4 Fulminant liver failure in Wilson's disease. Fulminant hepatitis in Wilson's disease usually develops on a background of already developed cirrhosis, as seen in this case. **A:** Cirrhotic nodules (N) are surrounded by inflamed fibrous septa with numerous bile ductular structures. The acute illness is related to progressive hepatocyte necrosis, inflammation and ductular reaction at the septal–parenchymal interface, as seen in the upper right field. **B:** Severe bile canalicular and hepatocellular cholestasis with both small- and large-droplet steatosis are present. **C:** Copper-binding protein is present in both periportal hepatocytes and sinusoidal Kupffer cells. (Explant liver, **A** and **B**: H&E; **C**: Victoria blue.)

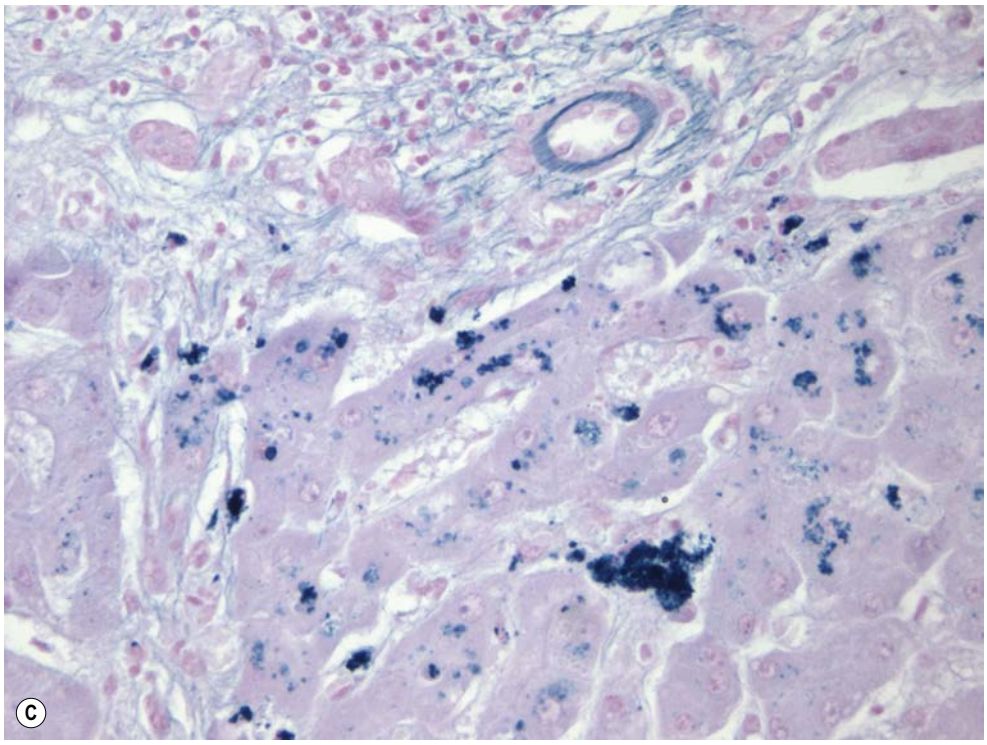

Fig. 14.4, cont'd

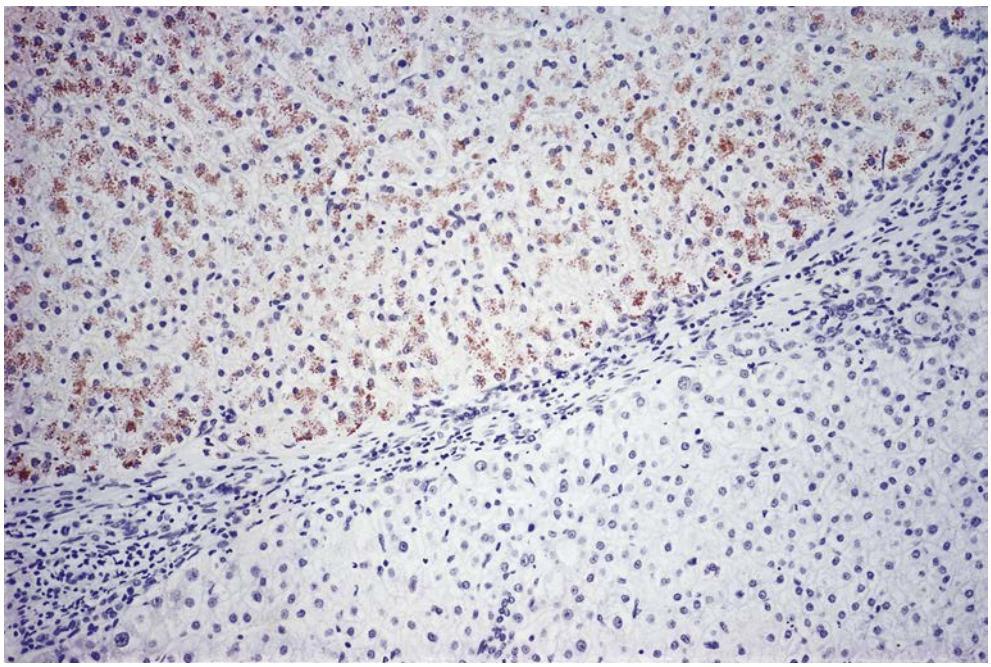

Fig. 14.5 Wilson's disease. The upper nodule is strongly positive for copper, stained orange-red. The lower nodule is completely negative. (Wedge biopsy, rhodanine.)

Because of the great variety of histological lesions in the liver, Wilson's disease can easily be mistaken for other liver disorders. Clinicians and pathologists should consider Wilson's disease in the differential diagnosis of hepatocellular disease, especially in the young, but also at all ages, including (uncommonly) older-aged individuals.[26] The disease can be arrested by treatment and its development prevented in siblings. The penalties for missing the diagnosis are therefore very great.

Iron overload

Siderosis

Siderosis (or haemosiderosis) means the presence of demonstrable iron in tissues, irrespective of cause. The main forms of iron in hepatocytes are ferritin, haemosiderin and haem.[27] Stainable iron is mainly haemosiderin, which is principally located in lysosomes and is seen as granules concentrated towards the biliary poles of the cells. Ferritin gives rise to more diffuse staining, imparting a bluish hue to the liver-cell cytoplasm on iron staining. Hepatocellular siderosis almost always shows a diminishing gradient of intensity from the periphery of lobules towards the central (efferent) veins. It is most severe in periportal regions (acinar zones 1) near small portal tracts, and least severe in centrilobular regions (acinar zones 3). The normal adult liver is usually negative on iron staining or at best shows minimal siderosis.[28] This is also true of the neonatal liver, although some cases may show mild periportal liver-cell siderosis (residual iron storage from the active period of hepatic haemopoiesis of the third trimester).[29]

Because iron stains of liver tissue are expected to be negative in most instances, a positive stain requires explanation. In this regard, two major categories of hepatic iron storage disease need to be considered, designated as **primary**[30a] **and secondary iron overload disorders**[30b] (**Box 14.1**). The *primary disorders*[30a] are predominantly forms of hereditary haemochromatosis in which genetic mutations alter iron homeostasis in the gastrointestinal tract and liver. The *secondary disorders*[30b] are acquired conditions in which increased iron in the liver is due to exogenous sources of iron, abnormal erythrocyte destruction or changes in iron absorption and distribution related to underlying liver disease. The pathologist may be able to suggest the reason for the siderosis, based on the distribution of the stainable iron. For example, in most of the primary iron overload disorders, such as classic *HFE*-related haemochromatosis, the excess iron is mainly hepatocellular. In thalassaemia both hepatocytes and macrophages are positive,

Box 14.1 Primary (genetic) and secondary (acquired) iron overload disorders

Primary

Type and standard name of hereditary haemochromatosis (HH)
(*Gene mutated*--protein product affected)

Type 1A* Classical *HFE*-associated HH
(*HFE*--HFE: C282Y/C282Y homozygous)
Type 1B *HFE* compound heterozygote
(*HFE*--HFE: C282Y/H63D)
Type 1C *HFE* S65C heterozygote
(*HFE*--HFE: S65C)

Type 2A Juvenile HH
(*HJV*—hemojuvelin)
Type 2B Juvenile HH
(*HAMP*—hepcidin)

Type 3 Transferrin receptor 2 HH
(*TFR2*--transferrin receptor 2)

Type 4A Ferroportin disease
(*FPN* [*SLC40A1***]—*ferroportin*: loss of function)
Type 4B Non-classical FPN disease
(*FPN*[*SLC40A1*]—ferroportin: gain of function)

Aceruloplasminemia
Others

Secondary

Transfusion
Haemolysis
Haemodialysis
Dietary
Underlying liver disease (e.g. chronic hepatitis, fatty liver)

**Most common type of hereditary haemochromatosis*
***The ferroportin gene* FPN1 *is also known as* SLC40A1

while exogenous iron overload leads to Kupffer-cell storage in the first instance. Various types of underlying liver disease are also associated with siderosis. Cirrhotic livers of varied aetiology may contain much iron,[31–33] even within macroregenerative nodules.[34] In viral hepatitis and alcoholic liver disease small amounts of stainable iron are often found. Siderosis in the setting of non-alcoholic fatty liver disease (**dysmetabolic iron overload syndrome**) is increasingly recognised.[35a,35b] Dense, iron-positive granules are common in endothelial cells in a variety of conditions, including acute hepatitis,[36] chronic hepatitis B and C[37] and alcoholic liver disease, but their significance is not known. As mentioned earlier in the chapter, siderotic livers rarely may also stain positively with rhodanine or other copper stain due to physiologic storage cuproproteins.[22b]

The siderotic liver should be evaluated for the **distribution of stainable iron** among the various cell types, the **grade of siderosis**, the **presence of any related tissue damage** (fibrosis, cirrhosis, necrosis or even hepatocellular carcinoma) and **coexisting liver disease of other aetiology**. Various numerical methods of assessing the degree of siderosis (discussed below) are also helpful in evaluating causation and the effectiveness of therapeutic iron removal.

Numerical assessment of tissue iron

Many different systems have been devised for the quantification of iron in tissue sections.[38] **Histological grading of hepatocellular iron** can be simply scored on a scale from 1 to 4, with grade 1 representing minimal deposition (recognisable only with a high-power objective), grade 4 massive deposits with obliteration of the usual lobular gradient, and grades 2 and 3 intermediate amounts. Examples are shown in various illustrations to this chapter. The alternative comprehensive grading system of Deugnier and colleagues[39a] measures iron not only in hepatocytes but also in mesenchymal cells, bile-duct epithelium, blood vessels and connective tissue. Kupffer-cell haemosiderin, by contrast, is not graded numerically, but its presence should be noted in the diagnosis (using modifiers such as 'diffuse', 'minimal' or 'mild' when necessary). The presence of Kupffer-cell siderosis is usually *a priori* evidence against classical (*HFE*-related) haemochromatosis, except for certain rare types (discussed later).

Hepatic iron concentration (HIC) can now be determined by magnetic resonance imaging[39b] or by measuring the iron concentration directly from a specimen of liver tissue. A separate biopsy core or larger tissue section can be embedded in paraffin and processed for iron quantification, or the concentration can be determined from a biopsy specimen obtained for histology or by fine-needle aspiration biopsy.[40] An actual paraffin block (biopsy, explant, postmortem) can be analysed[3] after histological examination is complete.[41] This has the advantage that the nature of the sample is known.[42] HIC has also been used in conjunction with the subject's age in order to calculate a hepatic iron index,[43] but its diagnostic value has been superseded by current diagnostic algorithms which include genetic testing, global assessment of serum iron indices and other parameters.[44]

Primary iron overload disorders

Molecular genetic studies have now defined a variety of heritable disorders affecting iron handling by the gastrointestinal tract and liver.[45] Several of these are listed in **Box 14.1**, and the reader is encouraged to consult the 'General reading' section at the end of this chapter for further details. The best understood of the primary iron overload disorders was first described in 1889 by von Recklinghausen[46] and is the disease referred to as 'hereditary haemochromatosis'. The majority of these cases are examples of what is currently known to be classic *HFE*-related hereditary haemochromatosis, which is discussed in the following section. However, the identical picture of predominantly periportal hepatocellular iron overload can be found in patients with various combinations of the gene defects listed in **Box 14.1**. There is thus a pathological pattern of **classic haemochromatosis** with more than one possible cause.[45,47]

Table 14.1 Characteristic diagnostic profile in *HFE* homozygous hereditary haemochromatosis.

Diagnostic modality	Typical result(s)
Serum transferrin saturation	>62% (screening threshold is >45%[46])
Serum ferritin	≥300 μg/L (men); ≥200 μg/L (women)
Hepatic iron concentration	>2200 μg/g dry weight (men) >1600 μg/g dry weight (women)
Hepatic iron index	≥1.9
Genetic testing	C282Y/C282Y
Liver biopsy	Hepatocellular iron ≥grade 2 No significant Kupffer-cell iron

Classic *HFE*-related hereditary haemochromatosis

This autosomal recessive disorder is associated with progressive accumulation of iron in the liver, heart, pancreas and other organs. The frequency of homozygous disease is approximately 1 person in 300,[48] while heterozygotes are found in about 1 person in 8–10.[49] Overt disease may be found in as few as 1 in 5000,[27] and even within families homozygous persons may show different rates of iron accumulation.[50] The ***HFE* gene**, the gene for this type of haemochromatosis, is located on the short arm of chromosome 6 at some distance from the HLA-A locus.[49,51–54] A missense mutation in *HFE* known as Cys282Tyr (C282Y) has been identified which results in tyrosine substitution for cysteine at position 282 of the gene protein product.[54] The majority (80%–100%) of individuals with the typical phenotype of hereditary haemochromatosis are homozygous for this mutation (designated C282Y/C282Y).[48,54] Genetic tests for C282Y can be performed on peripheral blood or on paraffin-embedded tissue.[55] Expression of the mutated HFE protein on duodenal crypt epithelium is one of several factors that have been considered important in the pathogenesis of iron overload in haemochromatosis.[56] A second mutation, His63Asp (H63D), has been identified in fewer patients with haemochromatosis, either in homozygous form or as compound heterozygotes in conjunction with C282Y (i.e. C282Y/H63D) or the wild-type (normal) protein.[54] In such cases, if stainable iron is present, it is usually only minimal or mild in periportal hepatocytes or in Kupffer cells, and may be due to concurrent liver diseases such as non-alcoholic fatty liver disease or chronic hepatitis.[57–59] Occasional patients with *compound heterozygous C282Y/H63D associated with other associated clinical disorders* such as alcoholic liver disease may undergo liver biopsy in order to assess iron overload and the degree of fibrosis. These cases usually show the same periportal-to-centrilobular decreasing gradient of haemosiderosis as C282Y homozygous haemochromatosis with a spectrum of hepatocellular haemosiderosis ranging from minimal to moderate (Grade 1–3 of 4). The degree of fibrosis is usually related to the underlying liver disease (e.g. alcoholic steatohepatitis, autoimmune hepatitis, chronic hepatitis C).[60] Other *HFE* mutations such as S65C (serine to cysteine) or rarer types are also reported.[61,62] Non-*HFE* hereditary haemochromatosis[30a] and other genetic disorders associated with iron overload are discussed later.

Until recently, a comprehensive panel of **diagnostic tests** combined with liver biopsy findings could be expected to provide a firm diagnosis of hereditary haemochromatosis (**Table 14.1**). However, the availability of genetic testing for *HFE*-related and other forms of haemochromatosis now sometimes obviates the need for liver biopsy, particularly if certain criteria indicate that the likelihood of hepatic fibrosis is low[63] [i.e. the patient is less than 40 years old, ferritin is less than 1000 ng/mL (<1000 μg/L), serum liver tests are normal and hepatomegaly is absent]. However, when there are coexisting liver diseases such as chronic

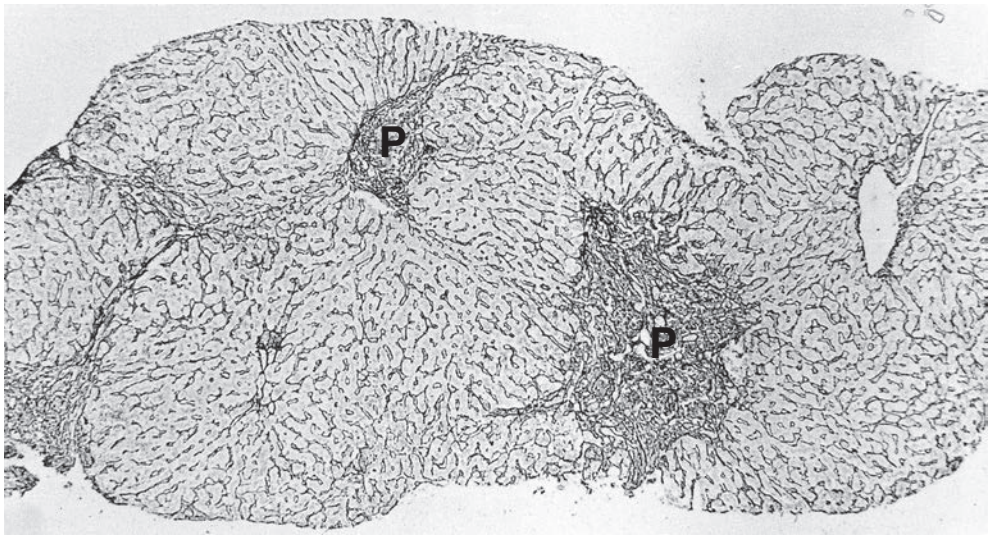

Fig. 14.6 Hereditary haemochromatosis. At this early stage of fibrosis, lobular architecture is still intact and vascular relationships are maintained. The portal tracts (P) are expanded by fibrous tissue. (Needle biopsy, reticulin.)

hepatitis C or alcoholism that may accelerate hepatic fibrosis in the presence of a genetic iron overload disorder[64] or there are other reasons for direct morphological assessment of liver tissue, liver biopsy continues to offer considerable information. Moreover, understanding the pathological progression of classic *HFE*-related hereditary haemochromatosis (discussed later) provides a useful comparative model of iron-related liver damage.

The first histological abnormality in homozygous *HFE*-related haemochromatosis is the appearance of stainable iron in periportal hepatocytes. This may be found incidentally in the course of investigation for other diseases. The unexplained presence of more than very small amounts of iron in hepatocytes should always raise the possibility of early hereditary haemochromatosis. The diagnosis can then be confirmed or refuted by means of genetic testing and/or calculating the hepatic iron index, as discussed previously. Early diagnosis is most important, because cirrhosis can be prevented by appropriate treatment both in patients and in their homozygous relatives, and life expectancy returned to normal.[65] In heterozygotes, stainable liver iron is either absent or very scanty.[46] From a practical standpoint, the presence of significant hemosiderin within sinusoidal Kupffer cells essentially excludes the diagnosis of classical *HFE* haemochromatosis (either homozygous or compound heterozygous).[47]

As iron stores increase, fibrosis begins to expand the portal tracts and slender septa extend from these to give a pattern of fibrosis resembling holly leaves (**Fig. 14.6**). The enlarged tracts contain iron-rich macrophages and a ductular reaction (which contributes to progressive fibrosis[66]), but usually show only mild or no inflammatory infiltration. Iron may be seen in the ductular structures and in the epithelium of interlobular ducts in small amounts; larger quantities are not found until a later stage, when parenchymal siderosis is severe. It is a challenging paradox that in early haemochromatosis most of the iron is in hepatocytes but there is little or no evidence of liver-cell damage, liver-cell function remains virtually unimpaired and the progressive lesion is portal in location. However, with increasing iron overload foci of sideronecrosis[39a] are found, comprising eosinophilic or lytic necrosis of iron-laden hepatocytes, often in close association with clusters of macrophages. The ratio of non-hepatocytic to hepatocytic iron, as assessed histologically, rises progressively. The ultrastructural progression of iron overload has also been examined.[67]

In fully developed hereditary haemochromatosis the lobular gradient of iron staining is obliterated; iron in hepatocytes is now seen throughout the lobules, whereas earlier it is more abundant in periportal and mid-zonal regions.[39a] Within individual hepatocytes the

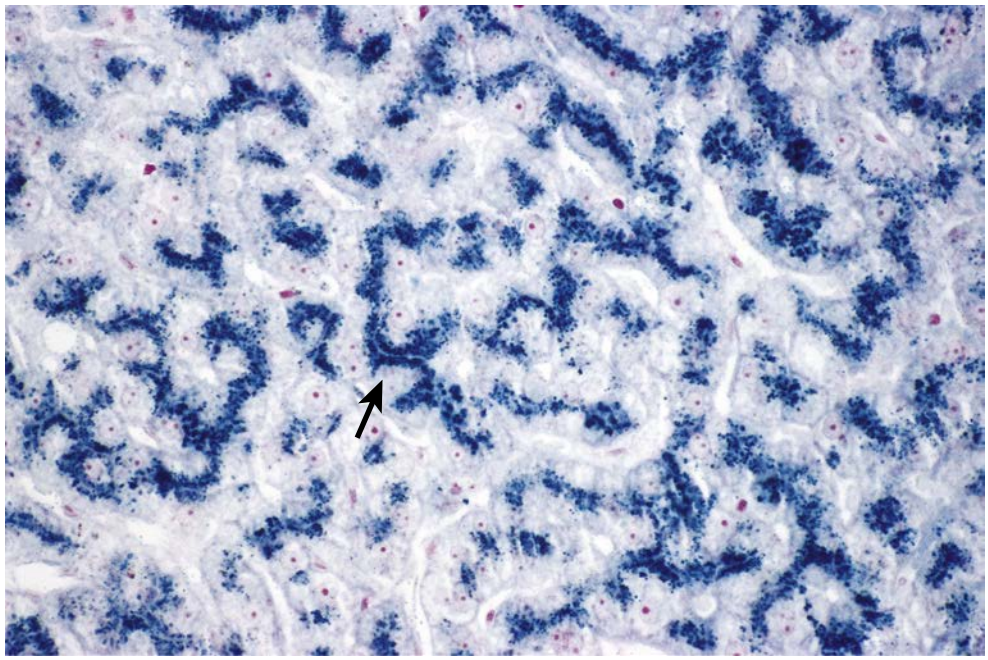

Fig. 14.7 Hereditary haemochromatosis. Grade 4 (maximal) liver-cell siderosis. Iron-rich granules in a pericanalicular location outline bile canaliculi (arrow). (Needle biopsy, Perls' stain.)

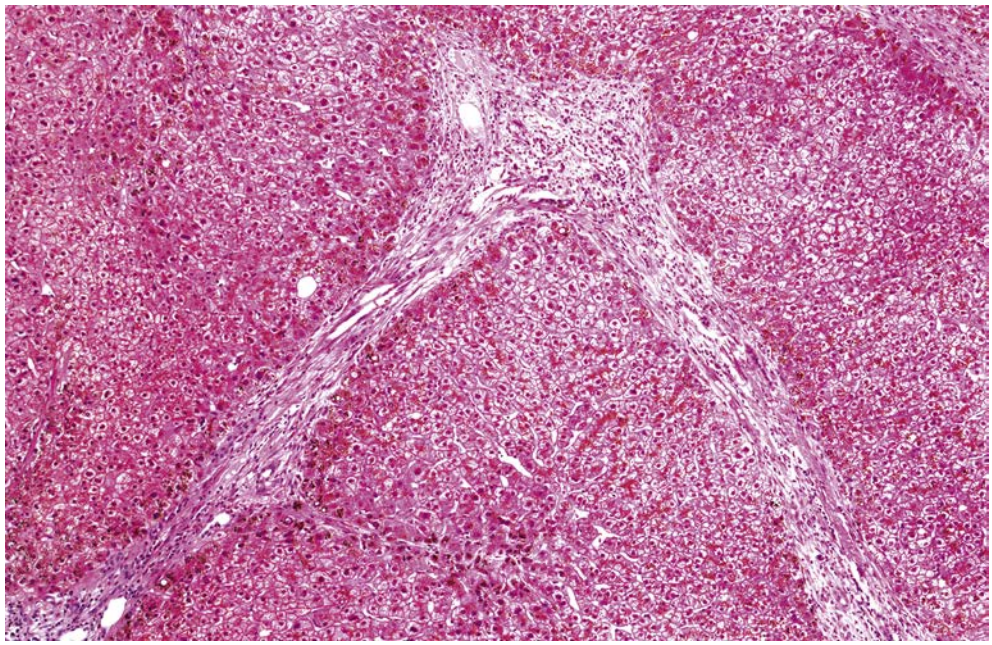

Fig. 14.8 Hereditary haemochromatosis. Fibrous septa surround irregular islands of liver parenchyma. (Wedge biopsy, H&E.)

iron is seen to be deposited in pericanalicular granules, outlining the bile canalicular system (Fig. 14.7). Cirrhosis slowly develops as fibrosis and hepatocellular hyperplasia alter the normal architectural relationships. True nodule formation is, however, a late event and for a long period there is fibrosis rather than cirrhosis, with irregular islands of parenchyma demarcated by fibrous septa (Fig. 14.8). The pattern is somewhat like that of chronic biliary tract disease. At this stage some regression of fibrosis as a result of treatment remains possible.[68] Once cirrhosis has developed, biopsy assessment of the effect of treatment on structural changes becomes more difficult because of a tendency for increasing nodule

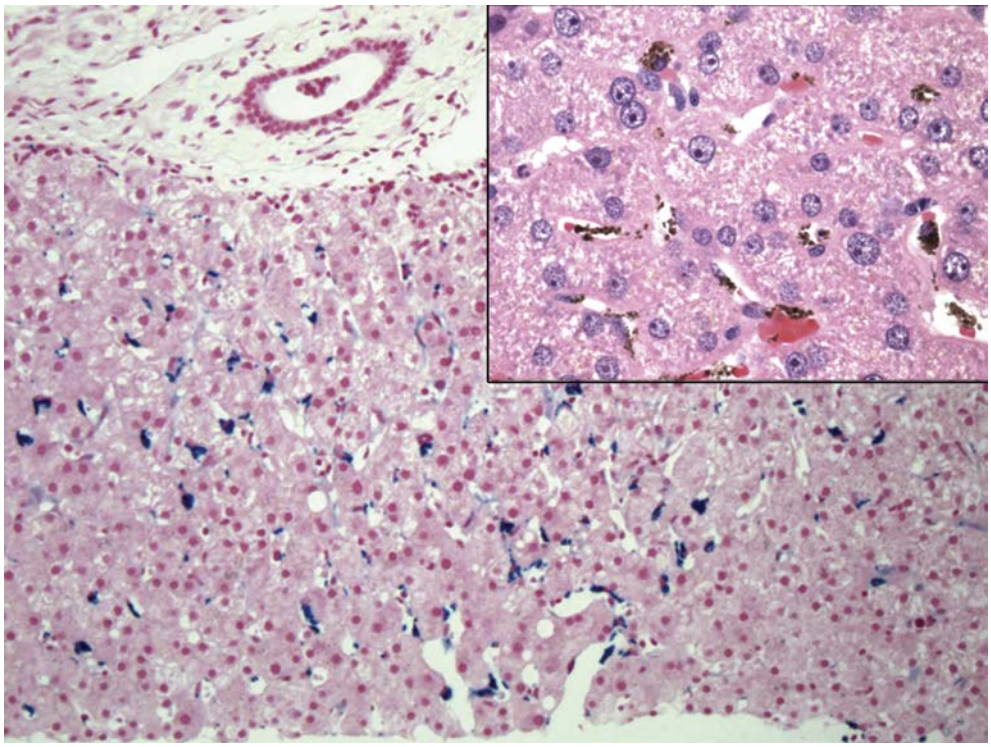

Fig. 14.9 Secondary (acquired) iron overload. Diffuse siderosis of sinusoidal Kupffer cells is present. Common causes are haemolysis, transfusions and haemodialysis. (Needle biopsy, Prussian blue iron stain.) Inset: Refractile brown haemosiderin granules are present within sinusoidal Kupffer cells. (Needle biopsy, H&E.)

size and compression or remodelling of septa. The onset of cirrhosis marks a fall in life expectancy and an increased risk of hepatocellular carcinoma.[65] The presence of iron-free foci may represent an early stage of malignant transformation.[69,70] Carcinoma has been recorded in non-cirrhotic patients with hereditary haemochromatosis, but is very rare.[69,71]

Effective treatment leads to a steady reduction in stainable iron. Iron encrusted onto portal collagen is usually the most resistant to removal and may be the only stainable iron remaining in the liver. Removal of iron unmasks a brown lipofuscin-like pigment in hepatocytes and connective tissue. Following liver transplantation for haemochromatosis, iron may reaccumulate in hepatocytes of the donor liver, but the rate is uncertain.[72]

Other primary iron overload disorders

Several types of **non-*HFE* haemochromatosis**[30a] (**Box 14.1**) and other genetic diseases such as **aceruloplasminaemia**[73] result in hepatic iron overload, with marked hepatocellular siderosis present in the majority. However, some of these diseases show an atypical iron distribution. Both early and later stages of **ferroportin-related iron overload** feature abundant Kupffer-cell siderosis[74,75] (in contrast to *HFE*-related haemochromatosis). Liver-cell haemosiderin is absent or minimal in the early stage, and as it progresses, it is seen throughout the lobule, without the usual gradient from periportal to centrilobular regions.[30a] The importance of ceruloplasmin in mediating egress of iron from cells is demonstrated in **aceruloplasminaemia,** where both hepatocytes and Kupffer cells accumulate haemosiderin.[73,76,77] Excessive Kupffer-cell siderosis that cannot be accounted for by one of the causes of secondary iron overload (see below) should therefore also raise the suspicion of a genetic iron overload disorder.

Secondary iron overload disorders

In routine practice most siderosis is secondary and located in sinusoidal Kupffer cells (**Fig. 14.9**). Haemolysis, transfusions and haemodialysis are common causes. Identification of

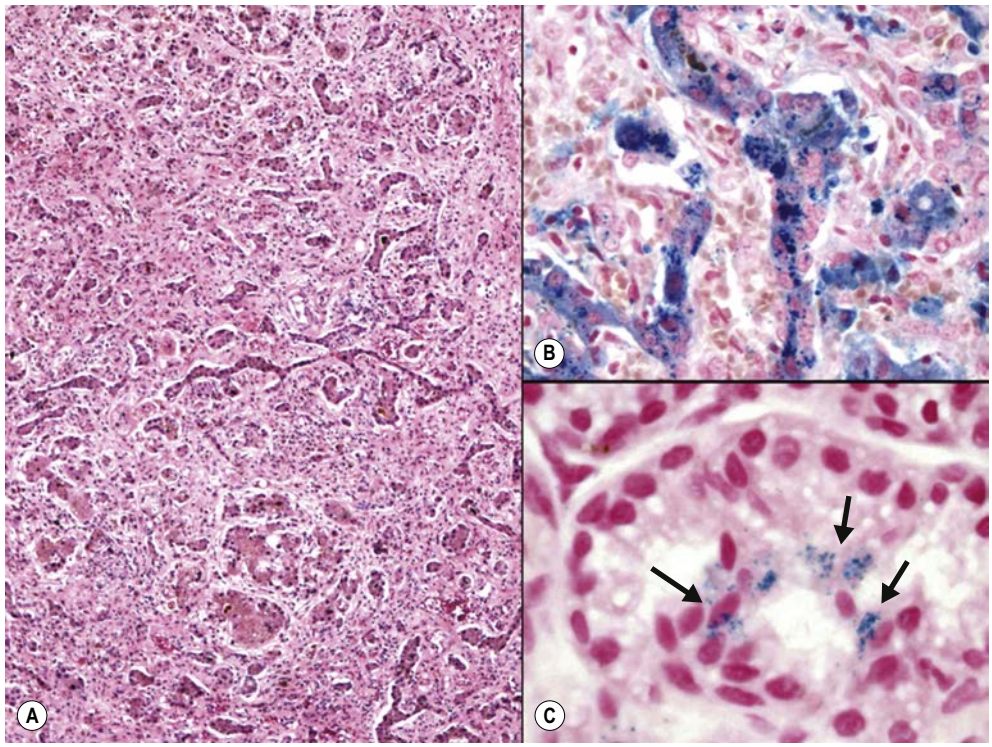

Fig. 14.10 Neonatal haemochromatosis. A: There is massive loss of liver parenchyma, with replacement by fibrosis and numerous bile ductular structures (ductular reaction). A few small clusters of remaining hepatocytes are seen in the lower half of the field. The pigment visible at this magnification includes both bile and haemosiderin. (Explant liver, H&E.) **B:** There is much haemosiderin within the bile ductular epithelium and in the few surviving hepatocytes, without significant Kupffer-cell siderosis. (Explant liver, Prussian blue iron stain.) **C:** Biopsy of the patient's labial salivary gland shows intraepithelial haemosiderin granules (arrows). (Needle biopsy, Prussian blue iron stain.)

significant iron overload with this distribution is evidence against most genetic forms of haemochromatosis, with the exception of ferroportin disease.[74] It is only when the threshold for macrophage iron storage is reached in such acquired disorders that liver-cell haemosiderin becomes evident in periportal regions (e.g. thalassaemia, sickle-cell disease).

Neonatal haemochromatosis

This severe liver disease of stillborn or newborn infants is characterised by marked liver injury and loss of functional parenchyma, a resultant acquired hepcidin deficiency and extensive siderosis of liver and extrahepatic organs (thyroid, pancreas, myocardium, minor salivary glands). It is not related to hereditary haemochromatosis in adults. Many cases are due to gestational alloimmune liver disease (GALD), in which maternal antifetal liver IgG antibodies cross the placenta, activate fetal complement and cause severe hepatocyte necrosis.[78–80] Postmortem and explant livers usually show cirrhosis (or exceptionally severe fibrosis with sparse, small regenerative foci), abundant ductular reaction, variable giant-cell transformation, cholestasis and very few remaining hepatocytes (**Fig. 14.10**). Many of the features resemble those seen in adults with acute liver failure and massive hepatic necrosis. Active Sonic hedgehog signalling by the ductular reaction in GALD mediates the development of extensive fibrosis.[81] Haemosiderin, when present, is limited to hepatocytes and the ductular reaction and is largely absent from Kupffer cells. The diagnosis may be confirmed by labial minor salivary gland biopsy[82] (**Fig. 14.10C**). The differential

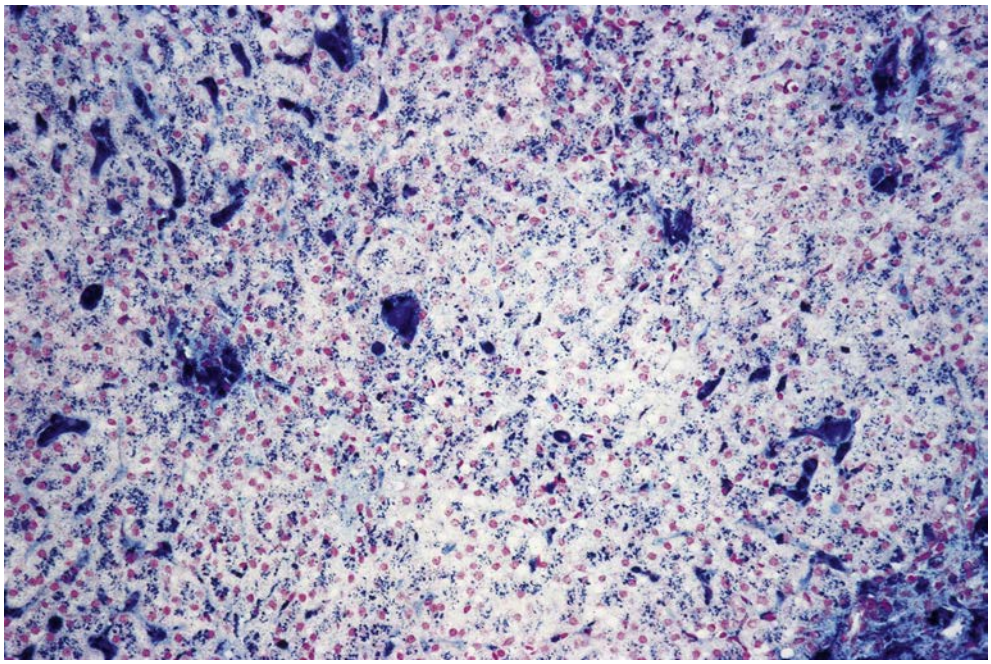

Fig. 14.11 Thalassaemia. In this example of secondary iron overload, hepatocytes show grade 3 siderosis. The darker clumps are iron-laden macrophages. (Needle biopsy, Perls' stain.)

diagnosis of neonatal haemochromatosis includes other causes of severe perinatal liver disease and liver failure such as mitochondriopathies and Down's syndrome with megakaryocytic transient myeloproliferative disorder.[80] A recent French multicentre retrospective study of many cases of neonatal haemochromatosis provides a wealth of clinical and pathologic data.[83]

Iron overload in haematological disorders

Siderosis is found in patients with thalassaemia and, less commonly, other haematological disorders. The iron overload is partly the result of blood transfusion. In addition to the hepatocytic siderosis, portal fibrosis and septum formation seen in hereditary haemochromatosis, there is iron in macrophages from an early stage (**Fig. 14.11**), and haemopoietic cells may be present. There is often more infiltration of portal tracts, septa and sinusoids by lymphocytes than in hereditary haemochromatosis (**Fig. 14.12**). This, together with focal hepatocellular damage in some cases, is attributable to transfusion-related hepatitis, usually hepatitis C.[84,85] The pattern of fibrosis and degree of inflammation in a liver biopsy often help to determine the relative roles of iron overload and hepatitis C in the progression of the disease. Kupffer-cell siderosis is a common finding in haemolysis, haemophagocytic syndrome,[86] haemodialysis and sickle-cell disease.[87]

Liver disease of varied aetiology

Chronic viral hepatitis, alcoholic and non-alcoholic fatty liver disease and cirrhosis of diverse aetiologies unrelated to hereditary haemochromatosis[31,32] are often associated with variable degrees of siderosis (**Fig. 14.13**). In **chronic hepatitis**, levels of serum iron and ferritin are sometimes increased as a result of release of iron from damaged hepatocytes, and iron may be seen on liver biopsy. The iron may be located in periportal hepatocytes, in Kupffer cells or in the endothelium of portal vessels.[37,88] Patchy iron-rich foci of hepatocytes in an

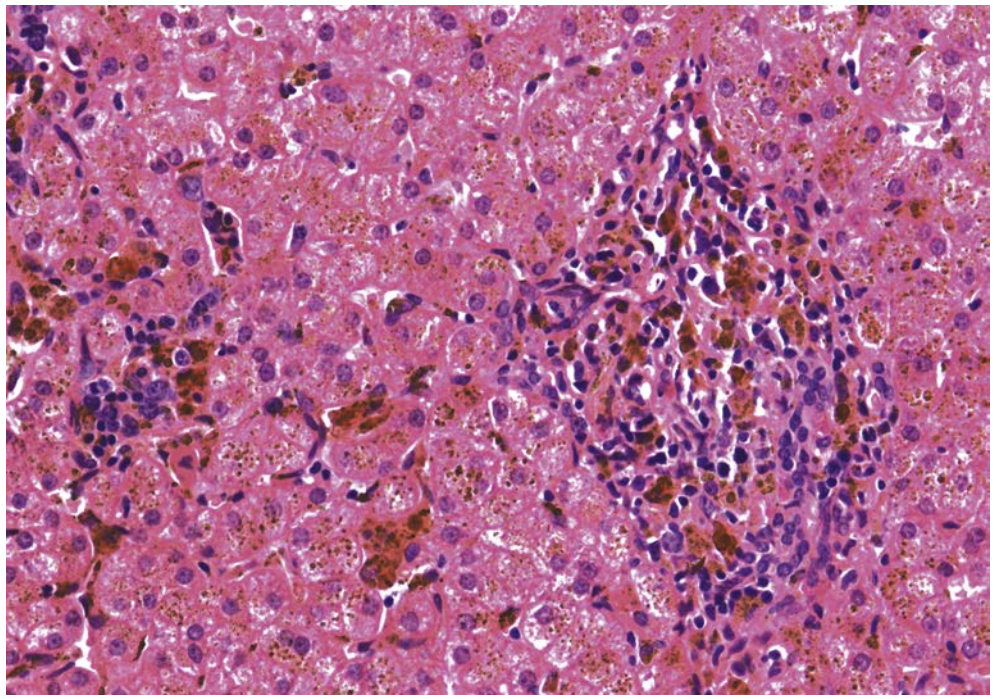

Fig. 14.12 Thalassaemia. There are iron-laden macrophages in the portal tract and in sinusoids. Haemosiderin granules are also evident in hepatocytes. The portal inflammation is probably due to transfusion-transmitted hepatitis C. (Needle biopsy, H&E.)

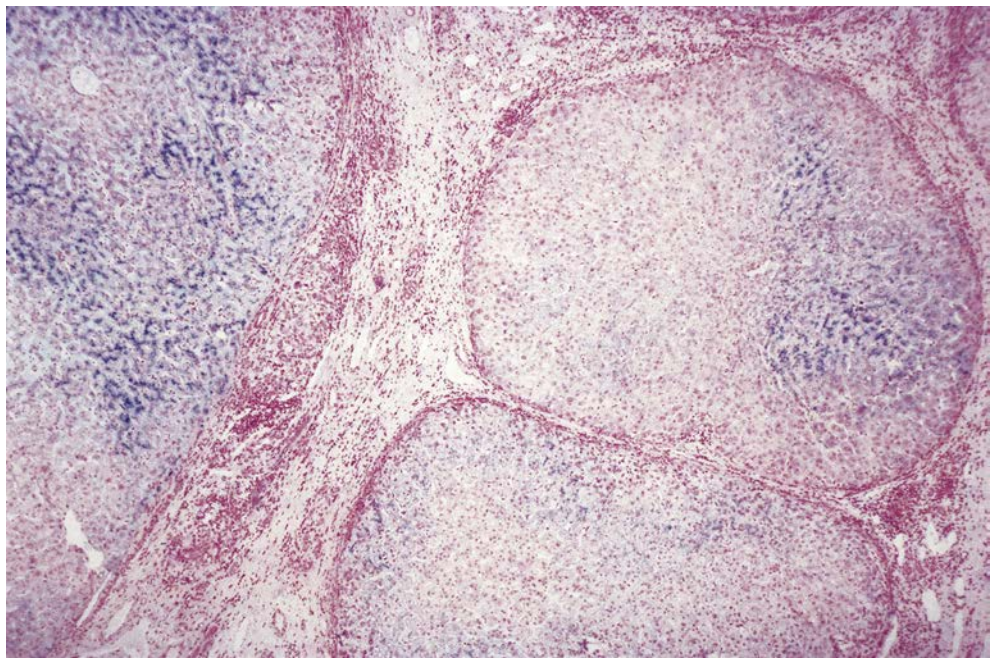

Fig. 14.13 Cirrhosis with siderosis. This case of relatively inactive cirrhosis due to chronic hepatitis C demonstrates considerable variability in the degree of hepatocellular siderosis among the nodules. (Explant liver, Perls' stain.)

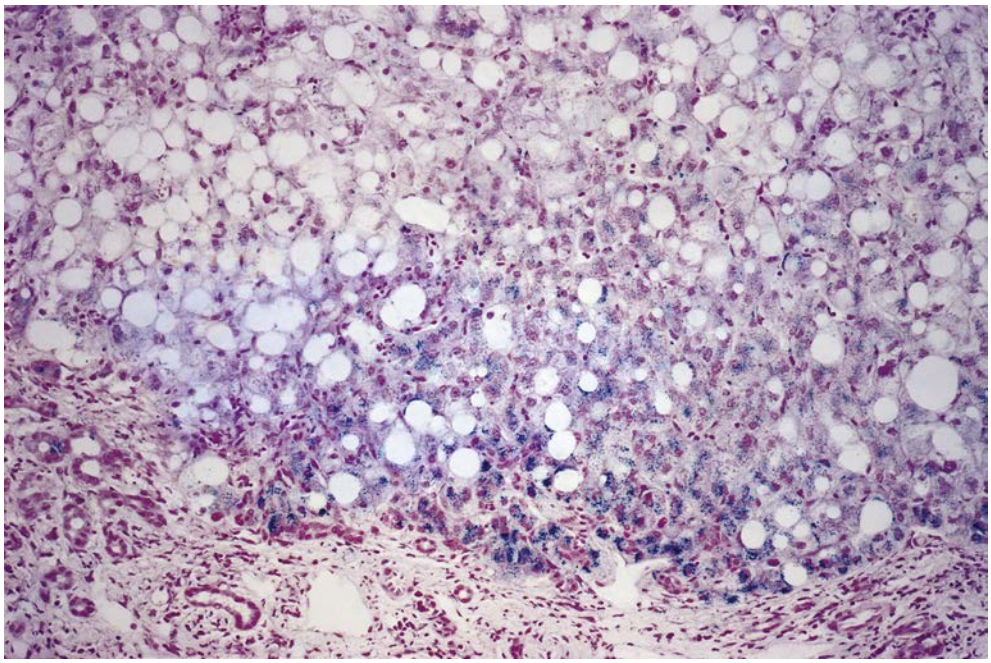

Fig. 14.14 Cirrhosis with siderosis. In this fatty cirrhosis in an alcohol abuser there is grade 2 hepatocellular siderosis, the cause of which needs investigation. (Needle biopsy, Perls' stain.)

otherwise non-siderotic biopsy may occasionally be seen.[89] In chronic hepatitis C, iron overload adversely affects therapy with interferon.[90] The severe siderosis which can complicate cirrhosis due to viral hepatitis and alcohol use may sometimes mimic hereditary haemochromatosis,[31,32] with marked elevations in HIC and hepatic iron index. In such cases there may even be siderosis of extrahepatic organs (heart, pancreas, stomach, thyroid, others).[91] Such cases require a comprehensive correlation of the histopathological features, biochemical test results, genetic analysis and other clinical data in order to clarify the aetiology of the iron overload. Biopsies from patients with **steatosis** sometimes show siderosis in periportal hepatocytes and in Kupffer cells, in which instance the possibility of **dysmetabolic iron overload** syndrome (DIOS; **see Fig. 7.12**)—associated with metabolic syndrome (central obesity, hypertension, hyperlipidaemia, hyperglycaemia and insulin resistance)—should be considered.[35a,92]

The presence of underlying liver disease is not in itself necessarily sufficient to explain the presence of hepatocellular haemosiderosis, nor does it preclude the diagnosis of a coexisting genetic iron overload disorder. An example of this is **porphyria cutanea tarda**, in which siderosis is present and increased frequencies of both hepatitis C virus infection[93,94] and *HFE* gene mutations have been identified.[94] Histological siderosis in **alcoholic liver disease** (**Fig. 14.14**) may reflect underlying homozygous or heterozygous haemochromatosis or concomitant spur-cell haemolytic anaemia.[95] Alcohol and chronic hepatitis C are known to accelerate the progression of liver disease in patients with *HFE*-related homozygous hereditary haemochromatosis.[64,96]

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CHAPTER 15

The Liver in Systemic Disease and Pregnancy

Introduction

Liver biopsies are often obtained to evaluate abnormalities of liver function tests in patients with known or suspected systemic disease and in the investigation of pyrexia of unknown origin.[1,2] In the latter, liver biopsy provides diagnostic information in approximately 15%–30% of cases.[3a] The hepatic changes associated with systemic diseases vary from obvious granulomas or steatosis (discussed in **Ch. 7**) to more subtle findings, such as an increase in liver-cell mitoses. The pathologist will want to know, whenever possible, whether or not the biopsy changes are specific for a systemic disease. For example, patients with *Turner syndrome* (karyotype 45,XO) often have abnormal liver tests (elevated aminotransferases and/or alkaline phosphatase) and a spectrum of lesions[3b,3c] ranging from nodular regenerative hyperplasia, multiple focal nodular hyperplasia, and cirrhosis to fatty liver disease, periductal fibrosis, or inflammatory hepatocellular adenoma,[3d] but these lesions are seen in many other clinical settings as well. In contrast, when granulomas are present, the differential diagnosis is more limited and their aetiology usually has important therapeutic implications. Liver biopsy in patients with acquired immune deficiency syndrome (AIDS) may demonstrate suspected hepatotoxicity due to antiretroviral drugs or hepatic involvement by a micro-organism already identified elsewhere in the patient, or may disclose a new diagnosis such as lymphoma. Liver biopsy also provides tissue for culture and special stains. This chapter examines the pathology of hepatic granulomas, hepatic changes in a variety of infectious diseases and liver involvement in gastrointestinal and haemopoietic diseases and the porphyrias.

In the unusual situation where liver dysfunction is found in pregnancy, the histopathologist may be called upon to differentiate intercurrent conditions such as viral hepatitis from several varieties of liver disease unique to pregnancy. This differential diagnosis is discussed later on in this chapter.

Granulomas

There are many causes of hepatic granulomas, including local irritants, infections, infestations and hypersensitivity to drugs. The constituents of these lesions, depending on the aetiology and inflammatory cytokines produced,[4] include large epithelioid cells, multinucleated giant cells, varied numbers of mononuclear cells and eosinophils. Hepatic granulomas can be further morphologically classified as **caseating (necrotising)**, **non-caseating**, **lipogranulomas** (**Ch. 7**) and **fibrin-ring granulomas**.[5–7] The causes vary in frequency from one country to another. Although the aetiology may be determined from the histological features, from special stains for micro-organisms, from culture of part of the biopsy specimen or polymerase chain reaction of the paraffin-embedded specimen[8] or from clinical and serological data, the cause of hepatic granulomas may remain unknown in some 10% to 36% of cases.[9,10]

From a practical point of view, biopsies containing granulomas fall into one of four groups:

1 The cause of the granuloma is seen under the microscope. Examples are the granulomas around schistosome ova, and the mineral-oil lipogranulomas found in portal tracts or near terminal hepatic venules.
2 The cause is not seen, but other histological features and clinical circumstances make the diagnosis clear. For example, granulomas near damaged bile ducts in a patient with clinically and immunologically typical primary biliary cholangitis are almost certainly due to this disease.
3 The cause is uncertain, but appearances favour one particular line of further investigation rather than another. For instance, sarcoidosis should be suspected when clusters of large granulomas with prominent epithelioid cells, large multinucleated giant cells and dense fibrosis are found in portal tracts.
4 The cause of the granulomas cannot be determined from the histological appearances. This is unfortunately common, and the help that the pathologist can then give to the clinician is limited.

These four circumstances can be summarised as **see the cause**, **know the cause**, **suspect the cause** and **don't know the cause**. Some of the histological guidelines for evaluating granulomas are summarised in **Table 15.1**.

Granulomas are found in up to 10% of liver biopsies, although recent studies suggest considerably lower percentages on the order of 2% to 5%.[11–14] They may be sparse, and suspicion of granulomatous disease is an indication for examining step sections from different levels of a paraffin block, if no lesions are seen initially. Because identifiable granulomas are generally more than 50 μm in diameter, serial sections 5 μm thick are unnecessary unless a single granuloma is to be further investigated.

Granulomas are commonly found in the liver in **sarcoidosis** and may even recur following liver transplantation.[15] The liver is usually one of several organs involved, but occasionally extrahepatic lesions are difficult to demonstrate and chest X-ray may be normal.[16] Liver

Table 15.1 Histological features of hepatic granulomas

Aetiology	Favoured site(s)	Special features
Sarcoidosis	Portal/periportal	Clustering Hyalinisation Inclusions in giant cells May destroy bile ducts
Tuberculosis	None	Necrosis
PBC	Portal	Near damaged bile duct Lobular granulomas uncommon
Drug	None	Eosinophils Other lesions often present (hepatitis, fat, cholestasis)
Mineral oil	Portal, perivenous	Oil vacuoles
Q fever, CMV, allopurinol, etc.	None	Fibrin-ring granuloma
CGDC	None	Brown pigment in macrophages May be necrotising
Cat-scratch disease, tularaemia, *Yersinia*	None	Purulent centre

CGDC, Chronic granulomatous disease of childhood; CMV, cytomegalovirus; PBC, primary biliary cirrhosis.

biopsy is helpful for diagnosis, especially in patients with fever and arthralgia.[17] The lesions may be found both in portal tracts and in lobules, and consist of well-defined, rounded granulomas with variable infiltration by inflammatory cells, including plasma cells and eosinophils (**Fig. 15.1**). The granulomas contain reticulin fibres (**Fig. 15.2**). Multinucleated giant cells may contain inclusions of different types.[18] Central necrosis may infrequently be present but is never as extensive as in tuberculosis. The granulomas often cluster in portal and periportal regions[19] (**Fig. 15.2**), and older lesions show dense hyalinised collagen. The fibrosis may extend to interfere with normal acinar structure, and in more severe cases may progress to cirrhosis.[18,20] A surprising degree of reactive portal and lobular inflammation may occasionally be seen in association with sarcoid granulomas, raising the question of concomitant hepatitis.[20] The lobular component consists predominantly of hyperplastic Kupffer cells; acidophil bodies are rare. The portal tracts show considerable variability in the amount of lymphocytic inflammation, and the most active portal inflammation is usually near granulomas. Serological tests for viral hepatitis should be obtained if there is serious diagnostic concern. In those few patients with sarcoidosis who develop portal hypertension,[21,22] it may be related to portal and periportal fibrosis or to broad areas of replacement fibrosis,[18] nodular regenerative hyperplasia[23] or cirrhosis.[18,20] Another rare complication of sarcoidosis is a primary biliary cholangitis-like lesion, with destruction of bile ducts and a clinical picture of chronic cholestasis.[24] Portal features suggesting biliary obstruction may also be present.[18,20] It should be noted that a diagnosis of sarcoidosis cannot be proved by histological examination of the liver alone, because very similar lesions are found in other granulomatous diseases.

In **chronic granulomatous disease of childhood,** defective neutrophil leukocyte function leads to the development of infective granulomas of different sizes, containing homogeneous eosinophilic material, necrotic debris or pus. Portal tracts are inflamed, and there may be fibrosis. A brown pigment of ceroid type accumulates in portal macrophages and to a lesser extent in Kupffer cells.[25–27] Abscesses and bile-duct fibroinflammatory lesions resembling primary sclerosing cholangitis are also seen.[28] The development of non-cirrhotic portal hypertension related to nodular regenerative hyperplasia and obliterative fibrosis of terminal venules and/or portal veins contributes to mortality.[29] **Common variable immunodeficiency** may be associated with portal and/or lobular epithelioid granulomas.[30]

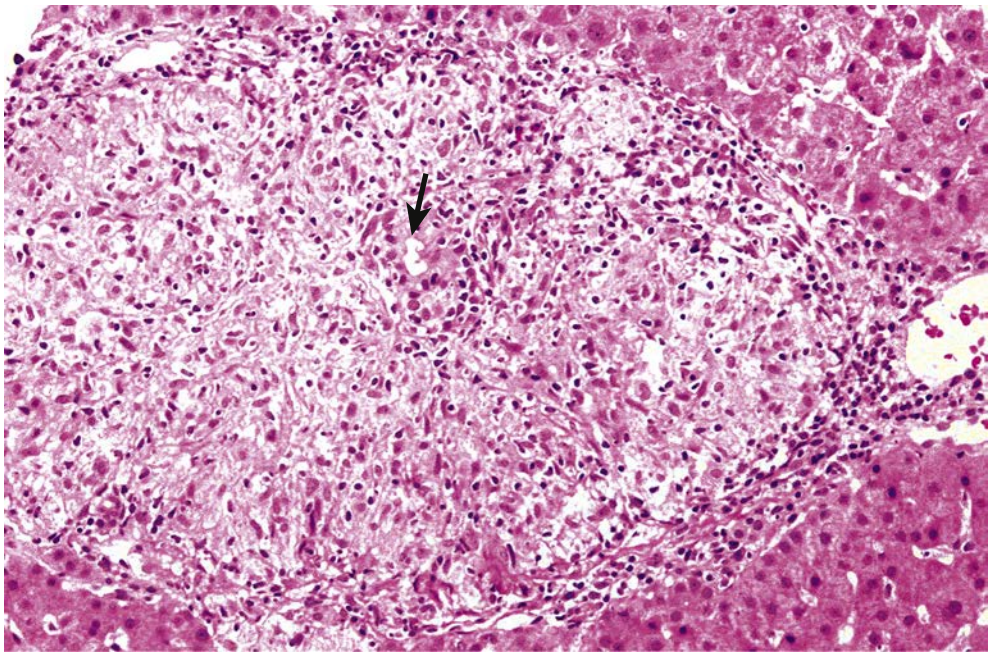

Fig. 15.1 Sarcoidosis. A cluster of epithelioid-cell granulomas with giant cells has expanded a portal tract and surrounded a bile duct (arrow). (Needle biopsy, H&E.)

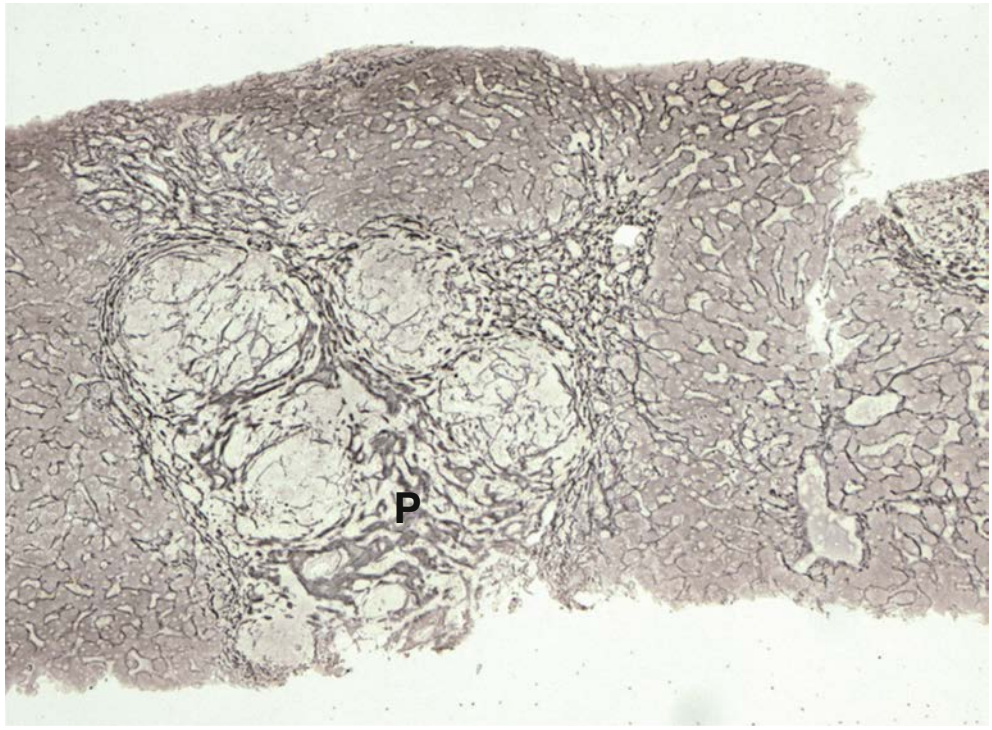

Fig. 15.2 Sarcoidosis. The granulomas are clustered in the portal tract (P) and periportal region, a characteristic feature of sarcoidosis. They are associated with increased reticulin fibres. (Needle biopsy, reticulin.)

Nodular regenerative hyperplasia (NRH) with elevated serum alkaline phosphatase levels appear to be the common hepatic findings in CVID. NRH or NRH-like lesions may recur if the patient undergoes liver transplantation.[31a] Nodular regenerative hyperplasia appears to be the most common hepatic lesion in CVID and is associated with elevated serum alkaline phosphatase,[31b] mild portal lymphocytic infiltrates with mild fibrosis[30,31c,32] and, rarely, primary biliary cholangitis or autoimmune hepatitis.[33]

A small number of patients with **chronic hepatitis C** may show non-caseating granulomas in the liver, either portal or lobular in location,[34,35] sometimes recurring after liver transplantation.[36] In one series, nearly 10% of granulomas were ascribed to this infection.[10] Their pathogenesis is unknown. In some instances, other causes such as schistosomiasis may become apparent during a thorough evaluation.[37] Necrotising granulomas at the edges of abscesses due to the Gram-negative bacillus *Achromobacter xylosoxidans* have been reported after cholecystectomy, with multilobulated 'coral-like' masses on computed tomography scan.[38]

Drugs and toxins should be considered in the evaluation of hepatic granulomas (**Ch. 8**), particularly if eosinophils are prominent.[39] A diverse array of particulate materials may cause granulomas, including aluminium,[40] feldspar[41] and silicone.[42] Biopsies with granulomas should therefore be examined under polarised light for evidence of particulate material. Dense reactive fibrosis may develop in the form of **sclerohyaline nodules** in individuals exposed to silica, chromium, cobalt or magnesium, either in the workplace or by intravenous drug abuse.[43]

The **fibrin-ring granuloma** is a distinctive though non-specific[44] form described in Q fever,[44–49] Hodgkin's disease,[50] allopurinol hypersensitivity,[51a] immune checkpoint inhibitor toxicity,[51b] cytomegalovirus (CMV)[52] and Epstein–Barr virus infections,[53] leishmaniasis,[54] toxoplasmosis,[50] hepatitis A,[55,56] giant-cell arteritis[57] and **systemic lupus erythematosus** (SLE).[58] This granuloma is composed of a fat vacuole surrounded by a ring of fibrin, epithelioid cells, giant cells and neutrophils (**Fig. 15.3**). Serial sections may be needed to demonstrate the typical fibrin-ring or 'doughnut' lesion.[46]

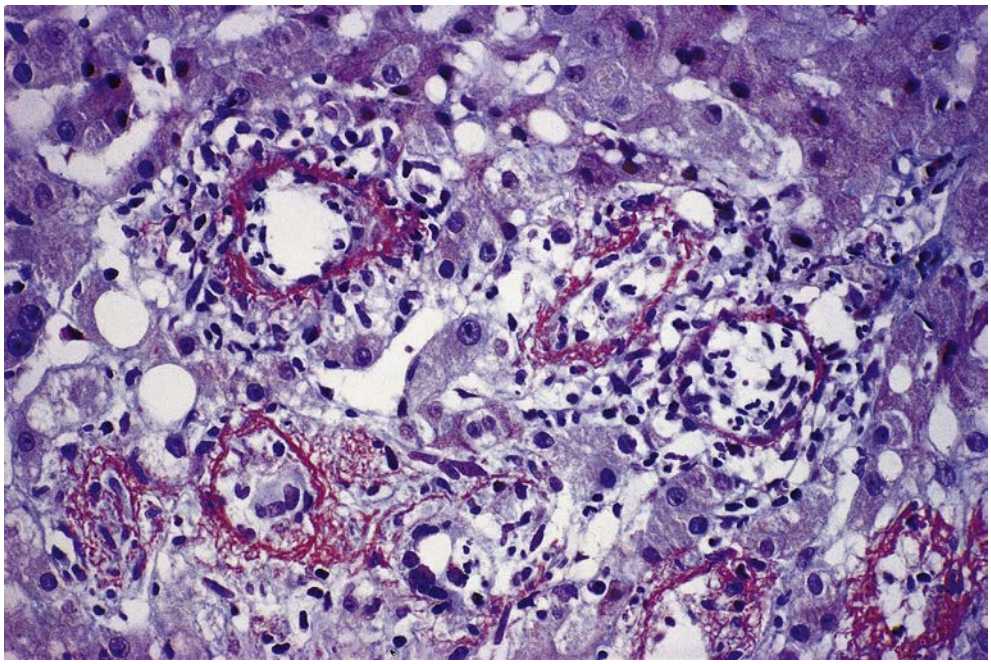

Fig. 15.3 Q fever. Small granulomas containing giant cells, fat vacuoles and neutrophil leukocytes are surrounded by rings of fibrin, stained red. (Needle biopsy, Martius scarlet blue.)

Simon and Wolff[59] described a syndrome characterised by fever, constitutional symptoms and hepatic granulomas, which does not respond to antituberculous drugs but improves on corticosteroid therapy or sometimes with methotrexate.[60] In some patients the syndrome resolves spontaneously without treatment.[61] The cause has not been established.

Viral diseases

The pathological changes in the liver resulting from virus infections other than hepatitis viruses have been reviewed by Lucas.[62] The viral haemorrhagic fevers, such as mosquito-borne **flavivirus** infection (**dengue fever**[63]) and rodent-borne **hantavirus** infections,[64] are characterised by mid-zonal or more extensive hepatic necrosis. In **yellow fever**, acidophil bodies are typically abundant; they were first described in this disease by Councilman over 100 years ago.[65,66]

Several viruses not normally associated with liver disease can occasionally cause liver damage. Examples include **herpes simplex virus** infection leading to irregular and randomly distributed areas of coagulative necrosis[67,68] (**Fig. 15.4**) and **adenovirus** infection.[69,70] In both infections, virus particles or antigens can be identified in hepatocytes. Paramyxovirus-like particles were described in adults with associated syncytial giant-cell hepatitis.[71] Multinucleated giant hepatocytes in liver biopsies from adults (**postinfantile giant-cell hepatitis**) may also be seen in hepatitis C virus mono-infection or co-infection with human immunodeficiency virus (HIV),[72] human herpesvirus-6A infection,[73] in autoimmune hepatitis and in other liver diseases.[74,75]

Cytomegalovirus infection

CMV has been implicated in some children with neonatal hepatitis (**Ch. 13**). Histological features include giant-cell formation as in other forms of neonatal liver damage,

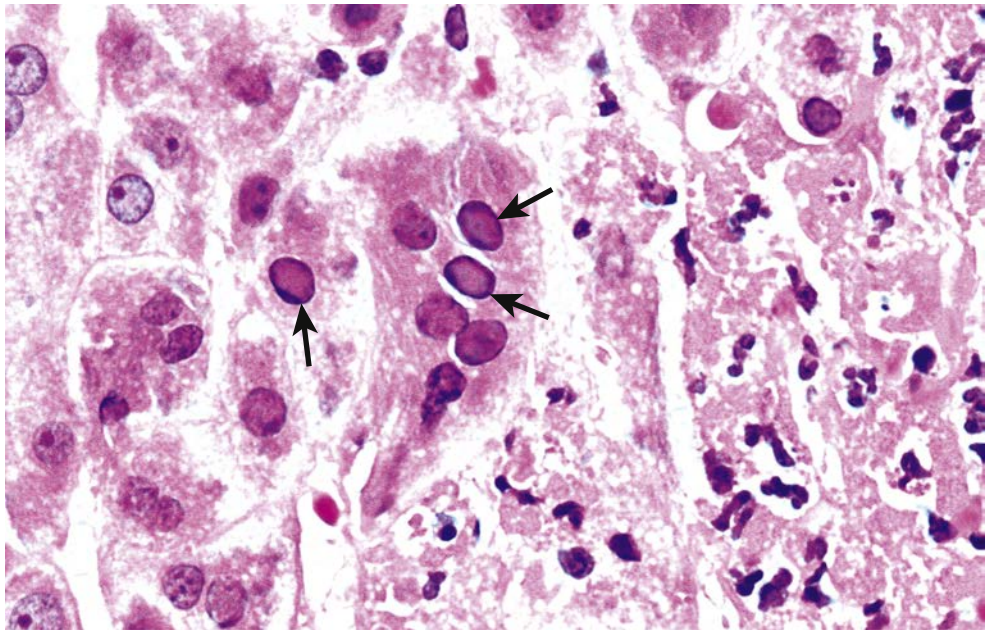

Fig. 15.4 Herpes simplex hepatitis. Pale, ground-glass-like intranuclear inclusions are present in a multinucleated hepatocyte (near centre) and elsewhere (arrows). An adjacent focus of necrosis with neutrophils is seen at the right of the field. (Needle biopsy, H&E.)

inflammation and cholestasis. Bile ducts are damaged and may be destroyed.[76] The CMV genome can be identified by the polymerase chain reaction in many cases.[77]

In later life, CMV infection can present as a mononucleosis-like illness, but also as hepatitis. Asymptomatic infection is common in immunocompromised patients. In these, the histological changes are often mild, but typical CMV inclusions are found in hepatocytes, bile-duct epithelium and endothelial cells (**Fig. 15.5**). Specific immunocytochemical staining reveals CMV antigens, even in cells without inclusions,[78] but sometimes with an abnormal granular basophilic cytoplasm.[79] Patients with CMV infection may also show aggregation of neutrophils in sinusoids, with or without evidence of CMV in neighbouring cells,[79] an important diagnostic consideration in immunocompromised patients or individuals who have received organ transplants. Larger accumulations of macrophages and lymphocytes can be seen, and epithelioid-cell granulomas have been reported.[80] In immunocompetent patients, there are varying degrees of focal liver-cell and bile-duct damage, portal inflammation, infiltration of sinusoids with lymphoid cells and increased mitoses in hepatocytes.[81] In such patients, it may not be possible to demonstrate CMV inclusions or antigen, a situation possibly analogous to hepatitis B virus infection, where inclusions and antigen may be scanty or absent during the acute attack while characteristic of the carrier state.[81]

Infectious mononucleosis

The liver is histologically abnormal in infectious mononucleosis even when there is no clinical jaundice.[82] Dense accumulations of atypical lymphocytes are found in portal tracts and sinusoids (**Fig. 15.6**). Sinusoidal aggregates must be distinguished from the more heterogeneous collections of cells found in extramedullary haemopoiesis. The infiltration also mimics that of leukaemia. Kupffer cells are enlarged. Epithelioid-cell granulomas are occasionally present.[12] Small foci of hepatocellular necrosis and acidophil bodies may be seen, but the diffuse hepatocellular damage characteristic of viral hepatitis is usually absent and extensive necrosis[83] is rare. Cholestasis is absent or mild.

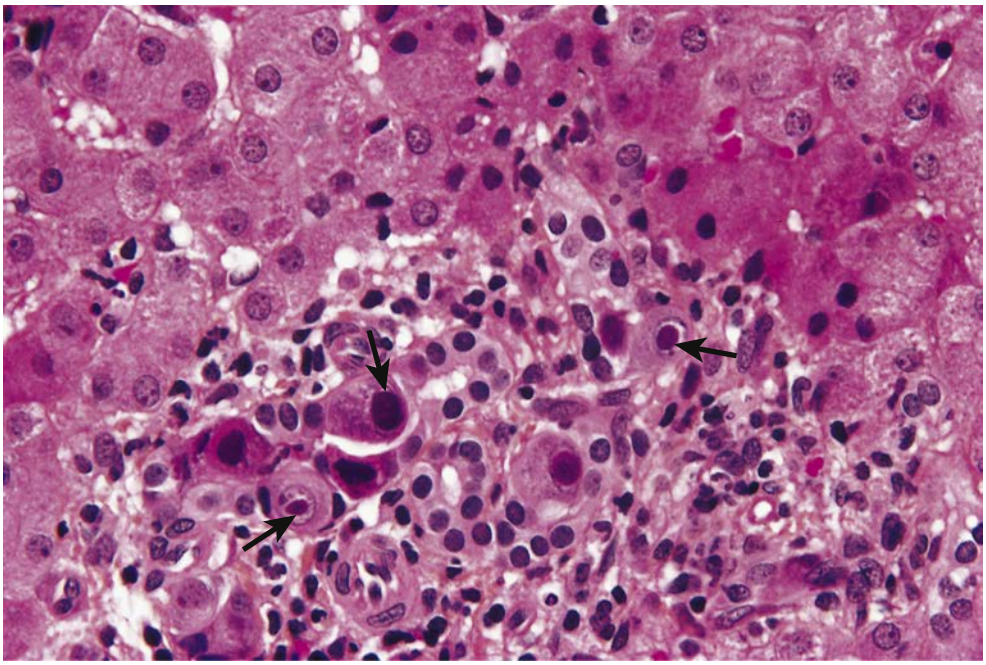

Fig. 15.5 Cytomegalovirus hepatitis in AIDS. Numerous cytomegalovirus inclusions (arrows) are seen within bile-duct epithelial cells. (Needle biopsy, H&E.)

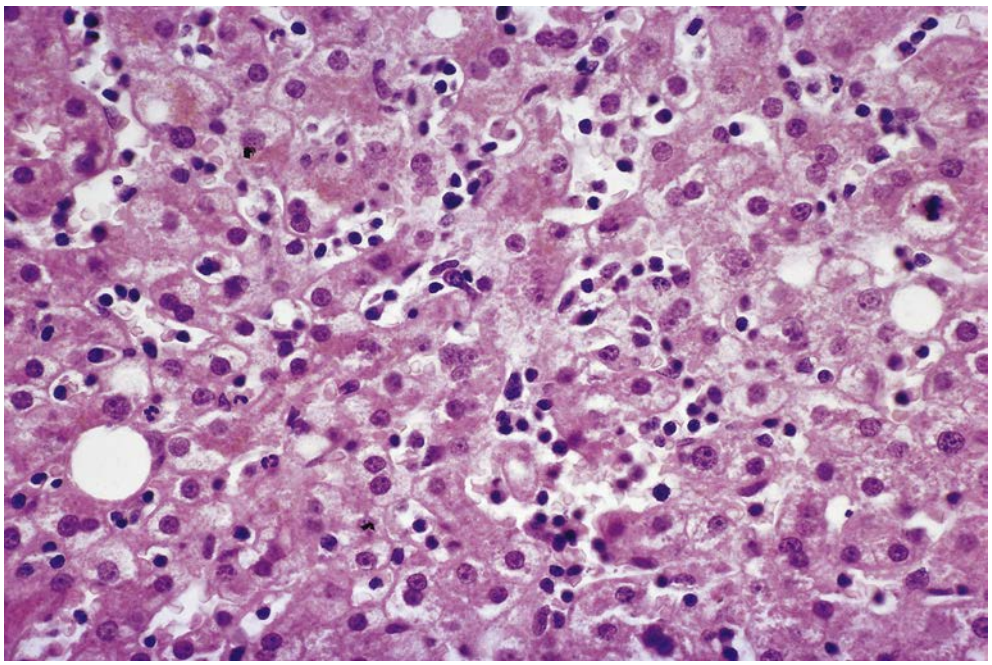

Fig. 15.6 Infectious mononucleosis. At left, a prominent sinusoidal 'beads-on-a-string' pattern is seen, consisting of atypical lymphocytes and hyperplastic Kupffer cells. Atypical lymphocytes are also present in the portal tract at right. (Needle biopsy, H&E.)

Acquired immune deficiency syndrome

A spectrum of hepatobiliary lesions has been associated with AIDS and HIV-1 infection since the onset of the epidemic[84–93] (**Table 15.2**). Liver biopsy continues to play an important diagnostic role in the evaluation of abnormal liver function tests in these patients,[86,87,94] particularly in managing the potential hepatotoxicity of highly active antiretroviral therapy

Table 15.2 Hepatobiliary lesions in HIV-1 infection and AIDS

Lesion	Cause(s) or type(s)
Granulomas	Mycobacteria, fungi, drugs
Abscesses	Staphylococci, streptococci, listeria
Bacillary peliosis	*Bartonella henselae*
Biliary tract disease (AIDS cholangiopathy)	CMV, cryptosporidia, microsporidia
Neoplasms	Kaposi's sarcoma, lymphoma, smooth-muscle tumours
Chronic viral hepatitis	HBV, HCV, HDV
Autoimmune hepatitis	Coexistent or following immune reconstitution
Other viral infections	CMV, herpes simplex virus, Epstein–Barr virus, adenovirus
Vascular lesions	Peliosis hepatis, sinusoidal dilatation
Drug toxicity	Sulpha agents, antiretrovirals
Miscellaneous	Steatosis, haemosiderosis, stellate cell hypertrophy, amyloidosis

AIDS, Acquired immunodeficiency syndrome; CMV, cytomegalovirus; HBV/HCV/HDV, hepatitis B/C/D virus; HIV-1, human immunodeficiency virus.

(HAART)[95–98] and the concurrent chronic hepatitis B and/or C, which may be present. Although Kupffer cells and endothelial cells[99–103] are potential target cells for HIV-1 infection, there are no specific hepatic lesions due to HIV-1, a few cases of alleged 'HIV-1 hepatitis'[104,105] notwithstanding.

Despite the reduction in morbidity and mortality due to antiretroviral therapy and prophylactic antibiotics,[106] opportunistic infections and neoplasms such as Kaposi's sarcoma and lymphoma must still be excluded on liver biopsy. Specimens should routinely be studied with acid-fast and silver stains for detection of high-incidence pathogens such as mycobacteria and fungi. Other methods such as Gram or Warthin–Starry stains can be applied, depending on the clinical and histological indications. A portion of the biopsy should be sent for culture.

Drug-related hepatotoxicity

Antiretroviral drugs may need to be excluded as the cause of liver dysfunction in HIV-positive individuals, particularly in those with negative hepatitis virus serology. Combination therapy frequently presents the problem of distinguishing among various medications. Some of the newer antiretroviral agents have been associated with elevated serum liver enzymes, but few morphologic data are available.[107] It is helpful to consider the type of hepatic damage reported with the several classes of **HAART agents**.[96] The nucleoside reverse transcriptase inhibitors cause mitochondrial damage and microvesicular steatosis, while the non-nucleoside reverse transcriptase inhibitors may produce hepatitis and confluent necrosis. The lesions attributed to protease inhibitors are various, including bile-duct damage, hepatocyte necrosis and ballooning, Mallory–Denk body formation, steatohepatitis and perivenular fibrosis.[96,97] Liver biopsy in some individuals receiving combined antiretroviral therapy shows coarse brown hepatocellular pigment granules resembling the pigment of Dubin–Johnson syndrome, often panlobular in distribution[108] (**Fig. 15.7**). The specific causative drug has not been identified. As antiretroviral liver injury is often idiosyncratic, the internet and other sources should be consulted for emerging descriptions of new cases.

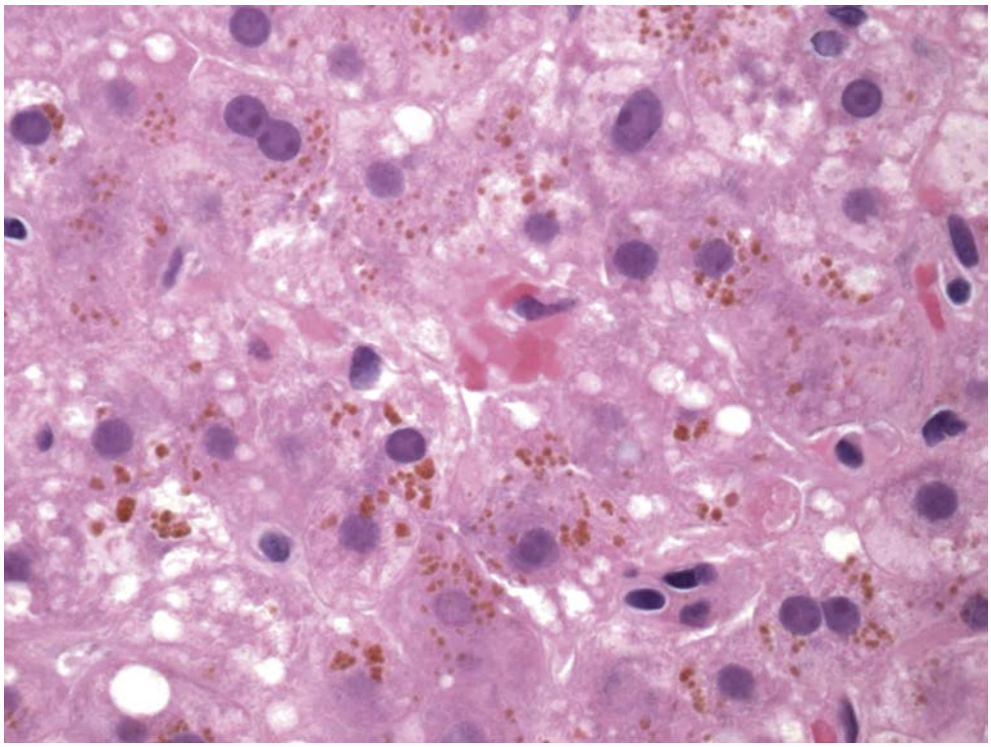

Fig. 15.7 Hepatocellular pigment associated with antiretroviral therapy for HIV. Hepatocytes show coarse brown pigment granules resembling Dubin–Johnson pigment. The pigment is often panlobular in distribution. The specific causative medication is not known. (Needle biopsy, H&E.)

Opportunistic infections and infestations

Opportunistic infections and infestations involving the liver and bile ducts in AIDS include *Mycobacterium avium–intracellulare* and *Mycobacterium tuberculosis* infections, CMV infection, cryptococcosis, candidiasis, histoplasmosis, leishmaniasis,[109] malaria, cryptosporidiosis[110] and microsporidiosis.[111–113] Mycobacterial and fungal infections frequently produce **granulomas**. *M. avium–intracellulare* results in numerous granulomas and the organisms are readily demonstrated by staining with diastase–periodic acid–Schiff (PAS) or the Ziehl–Neelsen method[114–117] (**Fig. 15.8**). Each granuloma consists of foamy histiocytes with few lymphocytes. The histiocytes often show a striated appearance on haematoxylin and eosin (H&E) staining due to the abundant packing of organisms in each cell. *M. avium–intracellulare* organisms are also well stained with Gomori methenamine silver. For screening of liver biopsies, particularly for *M. tuberculosis*, which may be present in fewer numbers than *M. avium–intracellulare*, the auramine–rhodamine fluorescent method[118,119] gives excellent results. Careful examination of special stains is of particular importance, as some AIDS patients have mycobacterial infection without typical granuloma formation; scant, single mycobacteria may be present within sinusoids or portal tracts. *Pneumocystis carinii* may disseminate to the liver, producing acellular exudative masses which closely resemble the pulmonary alveolar exudates.[120]

AIDS cholangiopathy

AIDS cholangiopathy resembles sclerosing cholangitis clinically and radiographically and is due to infections of the large bile ducts by several possible pathogens, including CMV, cryptosporidia and microsporidia.[111–113,121–123] Liver biopsy changes are those of large-duct obstruction. Cryptosporidia and microsporidia are best identified in aspirates obtained at endoscopy, duodenal biopsies or postmortem tissue samples of the major bile ducts.[111–113]

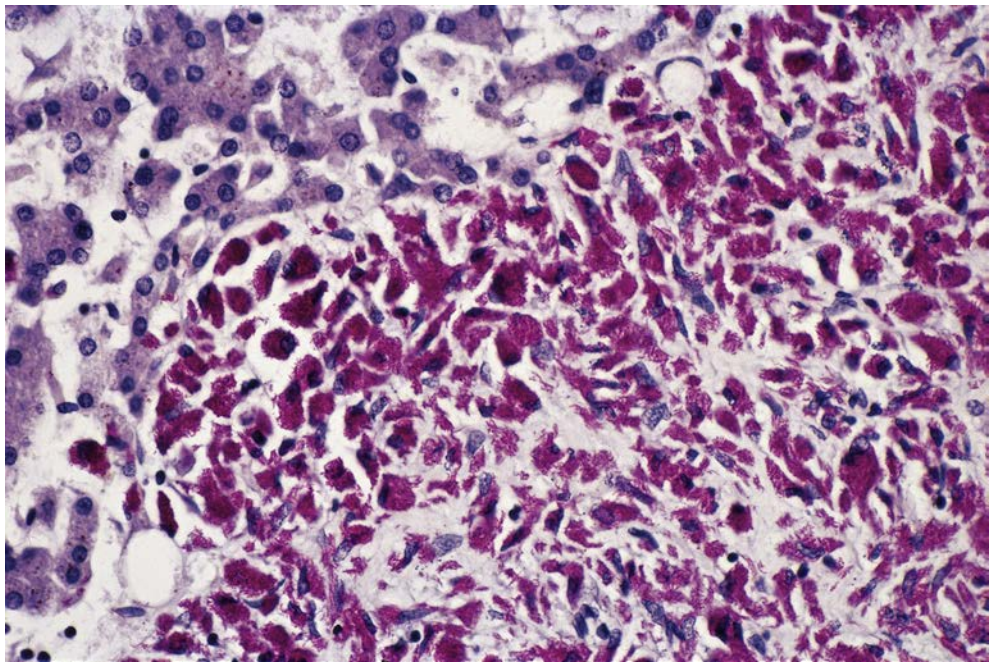

Fig. 15.8 ***Mycobacterium avium–intracellulare*** **in AIDS.** Abundant macrophages with densely packed mycobacteria are present within a granuloma. Individual organisms are best seen in the centre of the field. (Postmortem liver, Ziehl–Neelsen.)

Peliosis hepatis

Peliosis hepatis[116,124,125] in AIDS has been postulated to be due to endothelial damage by HIV-1 infection.[103] Alternatively, **bacillary peliosis hepatis** may develop as a consequence of hepatic infection by the Gram-negative bacillus *Bartonella henselae.*[126–129] Smudge-like or granular pink-to-purple material associated with a myxoid stroma is seen within dilated vascular spaces (**Fig. 15.9**), and the Warthin–Starry stain shows clumped bacilli in these areas.

Lymphomas

Lymphomas involve the liver as nodular masses or portal tract infiltrates (**see Fig. 7.3**) and are high-grade large-cell, immunoblastic and Burkitt types.[129,130]

Chronic hepatitis

AIDS patients have many of the same risk factors for infection by hepatitis viruses, and serum markers of prior infection or active viral hepatitis are often present. While the liver biopsy lesions of **chronic hepatitis B, C and delta** can vary considerably in persons infected with HIV,[131–133] it is now recognised that HIV infection may exert an adverse effect with accelerated progression of fibrosis.[134–137] Fulminant hepatitis may occur,[138] and in drug addicts a propensity for more severe chronic hepatitis with progression to cirrhosis has been noted.[139a] The long-term outlook for HIV-HCV coinfected individuals is likely to change in the future due to effective anti-retroviral therapy and direct-acting antiviral (DAA) treatment of HCV, unresolved questions of cost and access of DAA notwithstanding.[139b,139c] Coexistent autoimmune hepatitis may need exclusion when abnormal serum liver tests are found in the HIV-infected individual[139a] and rarely *de novo* autoimmune hepatitis may develop because of **immune reconstitution** after antiretroviral therapy has begun.[140]

Steatosis and other changes

Steatosis is common[141] and occasionally is periportal (**see Fig. 7.4**). Severe macrovesicular or microvesicular fat is cause for concern because this may reflect toxicity of antiviral

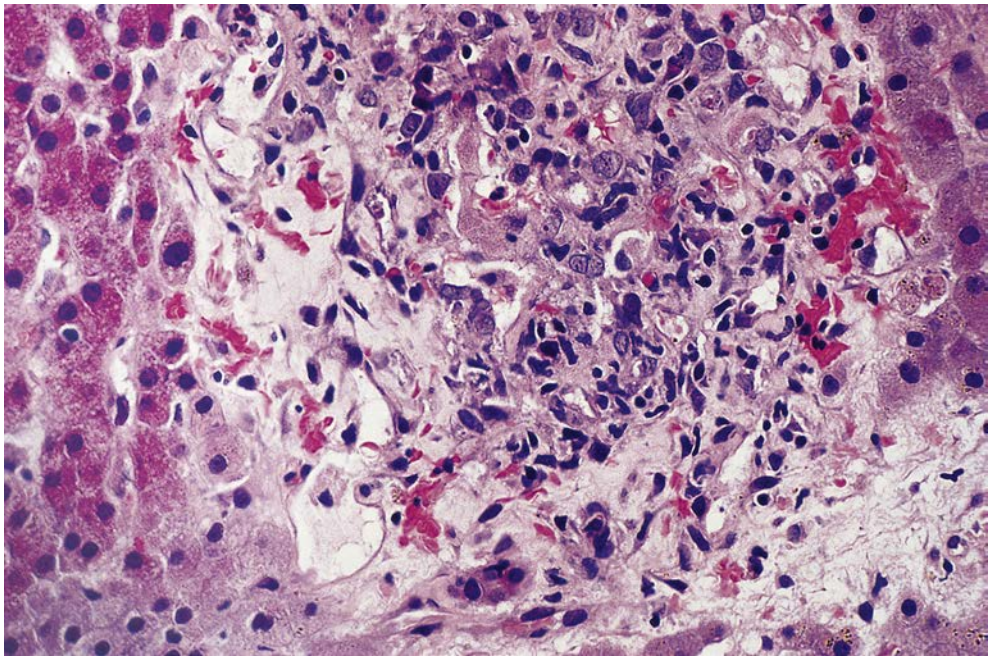

Fig. 15.9 Bacillary peliosis in AIDS. The portal tract is expanded by dilated blood vessels (left and right), chronic inflammatory cells and pink-grey smudge-like material (at centre) which contains bacilli. (Needle biopsy, H&E.)

medications[95,96,143,144] and can be associated with liver failure.[145] Non-alcoholic steatohepatitis (NASH) and abnormal liver enzymes may be present because of concomitant insulin resistance and features of metabolic syndrome.[142,146,147] **Siderosis** of Kupffer cells is related to transfusion or viraemia-associated erythrophagocytosis. In some cases, **non-specific changes** consisting of sparse portal or acinar lymphocytic inflammation with scattered apoptotic bodies are seen, with no apparent aetiology.

Other lesions reported include **nodular regenerative hyperplasia**,[148,149] **amyloidosis**[150] and **hypertrophied perisinusoidal stellate (Ito) cells** containing numerous lipid droplets.[151] In children, **giant-cell hepatitis**,[104,152] **chronic hepatitis** of uncertain cause[153] and **primary leiomyosarcoma**[154] are described.

Rickettsial, bacterial and fungal infections

Q fever

In Q fever, due to infection with *Coxiella burnetii*, liver involvement is common, although only a few patients present clinically with liver disease. Histological changes include focal necrosis, non-specific inflammation and fatty change. The most characteristic lesion is the **fibrin-ring granuloma**[44–50] (**see Fig. 15.3**), a granulomatous lesion is also seen in several other infections and in some patients taking allopurinol.[51a] Atypical lesions without annular arrangement or a central clear area (but containing irregular fibrin strands) are also found, as are non-specific granulomas without fibrin. In chronic Q fever, progressive fibrosis and cirrhosis have been reported.[155]

Brucellosis

In most patients with brucellosis, liver biopsy shows non-specific reactive changes comprising sinusoidal-cell hypertrophy, portal inflammation and focal necrosis.[156] Non-necrotising granulomas, often small and located within the acini, are more commonly found in the acute phase of the infection.[157]

Typhoid fever

Liver involvement is uncommon, but most patients with 'typhoid hepatitis' are jaundiced.[158] Liver biopsy shows a mild hepatitis with marked hyperplasia of mononuclear phagocytes, and lymphocytoid cells in sinusoids.[159] Characteristic granuloma-like collections of mononuclear cells, the typhoid nodules, are described.[160] Other features include fatty change and portal inflammation.[158,161]

Cat-scratch disease

Infection by a short Gram-negative rod, *B. henselae*, typically produces pyrexia and regional lymphadenopathy in children. Rarely, dissemination to the liver results in hepatic **granulomas with central stellate microabscesses** surrounded by palisaded macrophages, lymphocytes and an outer layer of fibroblasts.[162,163] The Warthin–Starry stain is used to identify the organisms.

Tuberculosis

Tuberculous lesions are present in the liver either as part of a generalised infection[164] or, less often, in the hepatobiliary form of the disease.[165] A normal chest X-ray does not exclude the diagnosis.[166] Granulomas are found randomly scattered in the parenchyma and also in the portal tracts. They range from small accumulations of macrophage-like cells to well-developed, large epithelioid-cell nodules with Langhans giant cells (**Fig. 15.10**). Central necrosis may or may not be present, and its absence does not exclude the diagnosis. Extensive necrosis (**Fig. 15.11**) is more likely to be seen when there are widely disseminated granulomas in the liver. Mycobacteria are seen in a minority of biopsies. Acute lesions contain little reticulin, while chronic ones undergo scarring. Remaining liver tissue shows non-specific reactive features and fatty change. Patients with AIDS sometimes have mycobacterial infection without typical granulomas, or may form tuberculous abscesses.[167] In all patients in whom tuberculosis is

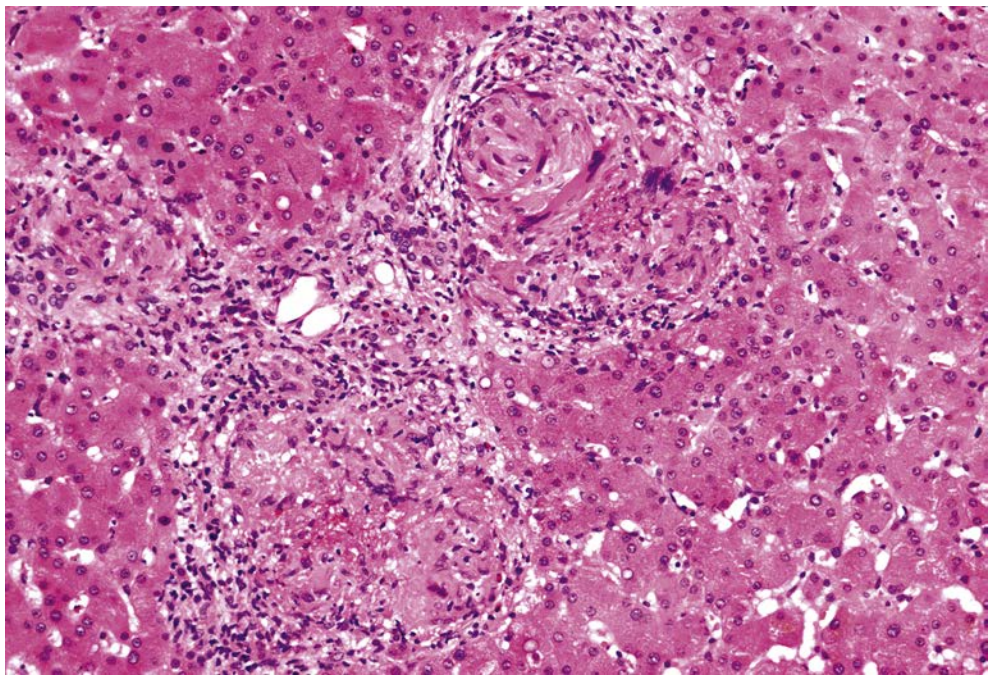

Fig. 15.10 Tuberculosis. Three parenchymal granulomas abut a portal tract. Multinucleated giant cells are visible in two of the granulomas. (Needle biopsy, H&E.)

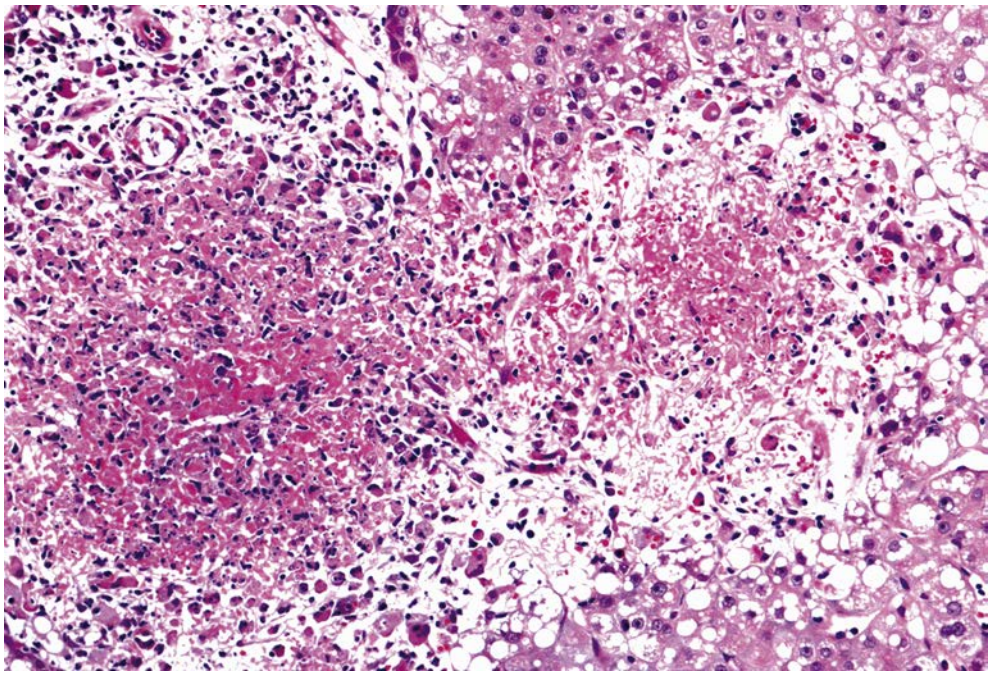

Fig. 15.11 Tuberculosis. There is extensive necrosis with little residual evidence of granulomas. Needle biopsy, H&E.

suspected, part of the liver biopsy specimen should be cultured. Polymerase chain reaction studies may also be performed on biopsy samples.[168] Lesions similar to those of tuberculosis have been reported in patients given BCG (bacille Calmette-Guerin) immunotherapy.[169–172]

Leprosy

In lepromatous leprosy, specific granuloma-like lesions composed of foam cells are found in the liver and often contain acid-fast bacilli.[173] Organisms are also seen in Kupffer cells. Epithelioid-cell granulomas of tuberculoid type, rare in lepromatous leprosy, are found in the livers of some patients with the tuberculoid form of the disease. Either type of granuloma is seen in borderline leprosy.[174]

Spirochaetal infection

Syphilis

In congenital syphilis, there is widespread fibrosis separating small groups of hepatocytes and spirochaetes are numerous. In early infections in adults, liver biopsies are normal or show non-specific changes.[175] Spirochaetes may be demonstrable histologically. In patients with secondary syphilis and jaundice or abnormal liver function tests, there is a variable degree of focal parenchymal inflammation, granuloma formation,[176] hepatocellular necrosis and portal inflammation. The portal reaction may mimic that of biliary obstruction,[177] and there may be inflammation of bile-duct epithelium as well as of the walls of small arteries and veins.[178,179a,179b] Because patients with syphilis often have other infections as well, lesions cannot always be confidently attributed to the syphilis itself.[180] The typical lesion of tertiary syphilis is the gumma, an area of necrosis surrounded by granulomatous tissue in which there is endarteritis. Healing is by fibrosis.

Leptospirosis

Most studies of the pathology of leptospirosis have dealt with autopsy material, in which disorganisation of liver-cell plates is a prominent feature. This is usually absent

from liver biopsies.[181] Hepatocytes are swollen, especially in perivenular areas, and there is an increase in mitotic figures. A few acidophil bodies and fat vacuoles may be seen. Kupffer cells are prominent, and there is a mild mononuclear-cell infiltrate in portal tracts. Cholestasis is common, and may persist after resolution of the other changes.[182] The diagnosis can be confirmed by demonstrating leptospiral antigen in paraffin sections by immunocytochemistry.[183]

Lyme disease

In hepatomegaly, elevated serum aminotransferase activity and biopsy features resembling viral hepatitis may be seen in patients infected with the tick-borne spirochaete *Borrelia burgdorferi*.[184] Liver-cell ballooning and numerous mitoses are accompanied by sinusoidal inflammation (hyperplastic Kupffer cells, lymphocytes, plasma cells and neutrophilic leukocytes). Rarely, necrotising granulomas with multinucleated giant cells and many eosinophils develop.[185] The organism can be identified in liver tissue by Dieterle silver stain.

Candidiasis

The most common hepatic manifestations of candidiasis in immunocompromised hosts are **microabscesses** and **granulomas**.[186,187] The more acute lesions show microabscess formation with central necrosis, visible on gross examination as 1–2-mm yellow-white nodules. Yeasts and pseudohyphae can be seen in some, but not all, cases with diastase–PAS and Gomori methenamine silver stains. The predominantly neutrophilic infiltrates are replaced by epithelioid histiocytes and granulomas as the lesions evolve, sometimes surrounded by reactive fibrosis. Candidiasis is most often diagnosed postmortem, but should be suspected in the presence of fever, abdominal symptoms and elevated serum alkaline phosphatase activity. Systemic candidiasis has been noted as an important cause of mortality in patients with zone 3 or multilobular hepatic necrosis due to **exertional heatstroke**.[188]

Histoplasmosis

Hepatomegaly is common in disseminated histoplasmosis due to *Histoplasma capsulatum*. The disease is very occasionally seen in countries where it is not endemic.[189] The liver may rarely be the only organ clinically involved.[190] Liver biopsy shows non-specific inflammation as well as granulomas which may be mistaken for the lesions of tuberculosis.[191,192] The organisms may be scanty or abundant, and are found in Kupffer cells and granulomas. They are round or oval, 1–5 μm across, and have a capsule and central chromatin mass. Diastase–PAS and other stains for fungi can be used for their demonstration and differentiation from Leishman–Donovan bodies; the latter are PAS negative in tissues.[193] Disseminated infection with *Histoplasma duboisii*, seen in Africa, also involves the liver. Nodular lesions contain the much larger and easily demonstrable organisms.[194]

Fibrous, calcified and even bony nodules are sometimes found in and deep to the liver capsule in long-standing histoplasmosis. The nodules, 1–3 mm in diameter, may have a necrotic core surrounded by granulomatous tissue, and the organism is demonstrable in some instances.[195]

The liver in sepsis

Hepatic changes in sepsis are the result of infection of the liver itself, of circulating toxins, of ischaemia or of a combination of these factors. In many patients the exact cause cannot be established.

Infective lesions include **liver abscess** and **bacterial cholangitis**. Less commonly, infection produces a diffuse bacterial hepatitis in which bacterial colonisation of the liver is associated with portal inflammation.[196] Infection in areas drained by the portal venous system can give rise to **pylephlebitis** (**see Fig. 12.3**). Rarely, cholangiographic and histological

features resembling primary sclerosing cholangitis develop in sepsis, possibly related to ischaemic damage to large ducts.[197] Postmortem liver sections from septic patients may show neutrophils aggregated within sinusoids and in sparse numbers dispersed throughout the connective tissue of portal tracts.

Patients with extrahepatic sepsis are often jaundiced, especially when the infection is due to Gram-negative organisms.[198] Three histological patterns have been described in such patients. The commonest is **canalicular cholestasis**, most severe in perivenular areas (see Fig. 5.2). This is associated with various degrees of Kupffer-cell activation, fatty change and portal inflammation, but usually little or no hepatocellular necrosis.[199]

The second pattern is one of **ductular cholestasis and inflammation**, sometimes referred to as 'cholangitis lenta'.[198,200,201] Bile ductular structures and canals of Hering at the margins of portal tracts are dilated and filled with bile, often in the form of dense, highly pigmented deposits, and neutrophils are seen within and around the affected ductules (**Fig. 15.12**). Perivenular cholestasis is usually present, and periportal canalicular bile is also sometimes evident. These changes are not seen in uncomplicated bile-duct obstruction. They are common in the terminal stages of fatal acute or chronic liver disease complicated by sepsis. Damage to bile-duct epithelium has been reported,[202] but in most instances the interlobular bile ducts are not affected. Patients with the ductular cholestasis pattern have disproportionately elevated serum bilirubin levels compared with alkaline phosphatase and aminotransferases.[203] Because of its dire implications, this biopsy finding should be communicated rapidly to the clinician and sepsis should be investigated.

The third pattern is **non-bacterial cholangitis**, seen in **toxic-shock syndrome.**[204] The histological features are similar to those of bacterial cholangitis, but the biliary tree is anatomically normal, and the lesion is attributed to a circulating staphylococcal toxin rather than to bacteraemia. In many, but not all, patients the underlying lesion is a staphylococcal vaginitis associated with the use of tampons.

Parasitic diseases

Toxoplasmosis

Toxoplasma gondii is occasionally responsible for neonatal liver injury. In adults, hepatic changes include extensive lymphocytic infiltration of sinusoids, evidence of mild liver-cell damage and granuloma formation.[12,205] Trophozoites may be seen within necrotic hepatocytes and can be identified by specific immunocytochemical methods.[206,207]

Malaria

In non-immune patients with malaria there is hypertrophy of Kupffer cells, and these contain malarial pigment (haemozoin) in the form of fine, dark brown, or black pigment granules (**Fig. 15.13**). In acute malaria due to *Plasmodium falciparum*, they also contain erythrocytes, parasites and iron. Malarial pigment closely resembles schistosomal pigment. It often gives pinpoint birefringence and, like formalin pigment, is soluble in alcoholic picric acid. This distinguishes it from carbon, with which it may be confused.[208] Other black pigment in Kupffer cells, portal tract macrophages or granulomas can be seen after gold salt therapy or following knee or hip replacement with titanium-containing prostheses.[209] Following an attack of malaria, the pigment clears from the acini but can be found in portal macrophages.

The **tropical splenomegaly syndrome** (**hyperreactive malarial splenomegaly**) probably represents an abnormal immune response of the patient to the malarial parasite.[210] Large numbers of small T lymphocytes are seen in dilated hepatic sinusoids (**Fig. 15.14**). Kupffer cells are enlarged but hepatocytes remain normal. Malarial pigment is scanty or absent. The differential histological diagnosis is from leukaemia, hepatitis C virus infection, infectious mononucleosis, CMV infection and toxoplasmosis.

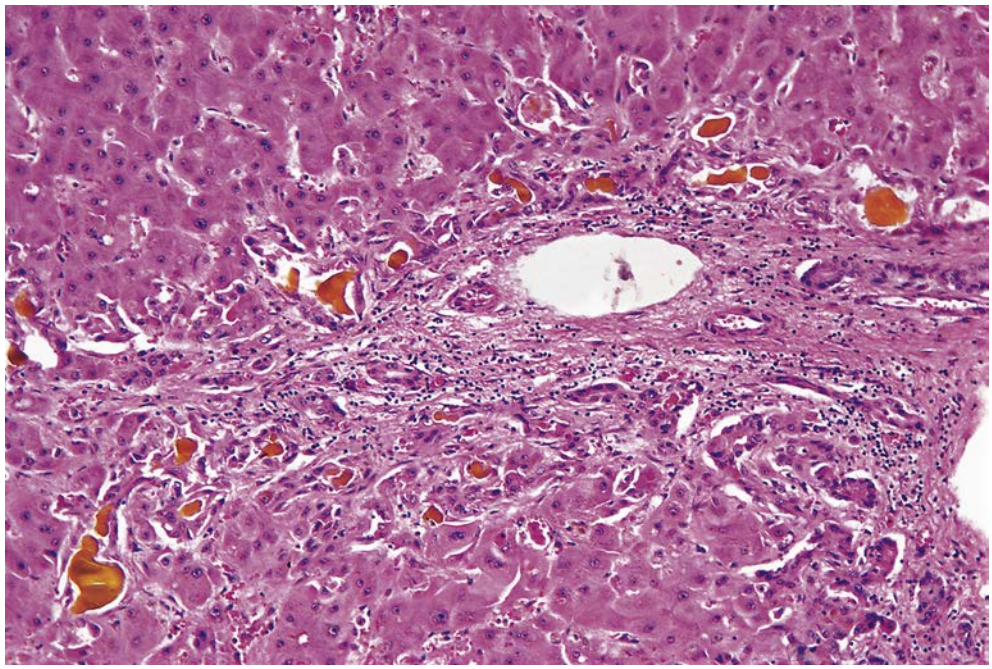

Fig. 15.12 Bile ductular cholestasis in sepsis. Proliferated bile ductules at the edge of the portal tract contain inspissated bile. The patient died of septicaemia. (Postmortem liver, H&E).

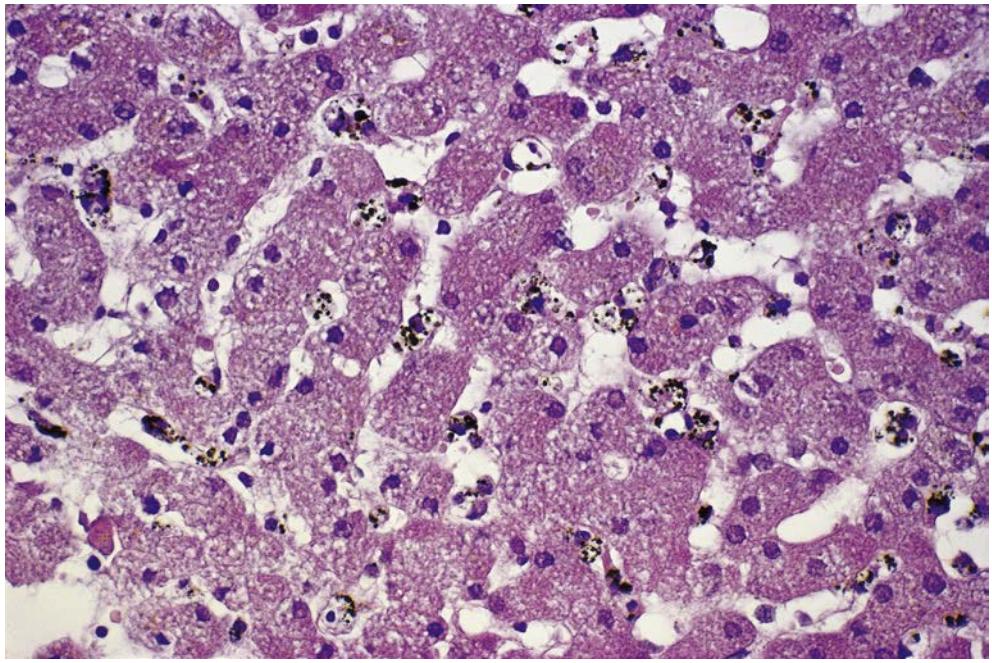

Fig. 15.13 Malaria. Kupffer cells contain abundant dark granules of malarial pigment. (Needle biopsy, H&E).

Visceral leishmaniasis (kala-azar)

Infection by *Leishmania donovani* produces striking hypertrophy of Kupffer cells and portal macrophages. These cells contain variable, sometimes very large numbers of Leishman–Donovan bodies, easily visible in H&E-stained sections (**Fig. 15.15**). The PAS stain after diastase digestion is negative, in contrast to the positive staining obtained with *Histoplasma*. In some patients the liver contains epithelioid-cell granulomas, which heal by fibrosis.[12,211]

Amoebiasis

In patients with liver abscesses due to *Entamoeba histolytica*,[212] the amoebae may be found at the margins of the lesion or, less often, within the necrotic debris. They may also be seen in the adjacent liver tissue. They are most easily demonstrated by the PAS or Giemsa methods. Organisms may be identified in fine-needle aspiration biopsy specimens[213] (**Fig. 15.16**).

Schistosomiasis

Liver lesions are usually caused by *Schistosoma mansoni* or *Schistosoma japonicum*, and less commonly by other species.[214] In acute schistosomiasis due to *S. mansoni*, the portal tracts are infiltrated by eosinophils, lymphocytes and macrophages. Kupffer cells are enlarged and there is focal hepatocellular necrosis. Granulomas around ova are rare.[215]

More commonly schistosomiasis is chronic. Ova, initially containing live miracidia, are trapped in portal tracts where they excite a granulomatous reaction. This is composed of epithelioid cells, multinucleated giant cells, eosinophils and lymphocytes (**Fig. 15.17**). Healing is by fibrosis. When ova are scanty and granulomas are no longer seen, step sections may need to be searched. Ziehl–Neelsen staining is then helpful, because the ova of species other than *Schistosoma haematobium* are acid-fast.[216] Schistosomal pigment, found in portal tracts in some patients with chronic or past schistosomiasis, is a fine, dark granular material closely resembling malarial pigment.

There are lesions in portal-vein branches of all sizes.[215] The smallest contain ova and granulomas. Angiomatoids, wide, irregular thin-walled vascular channels, are characteristically

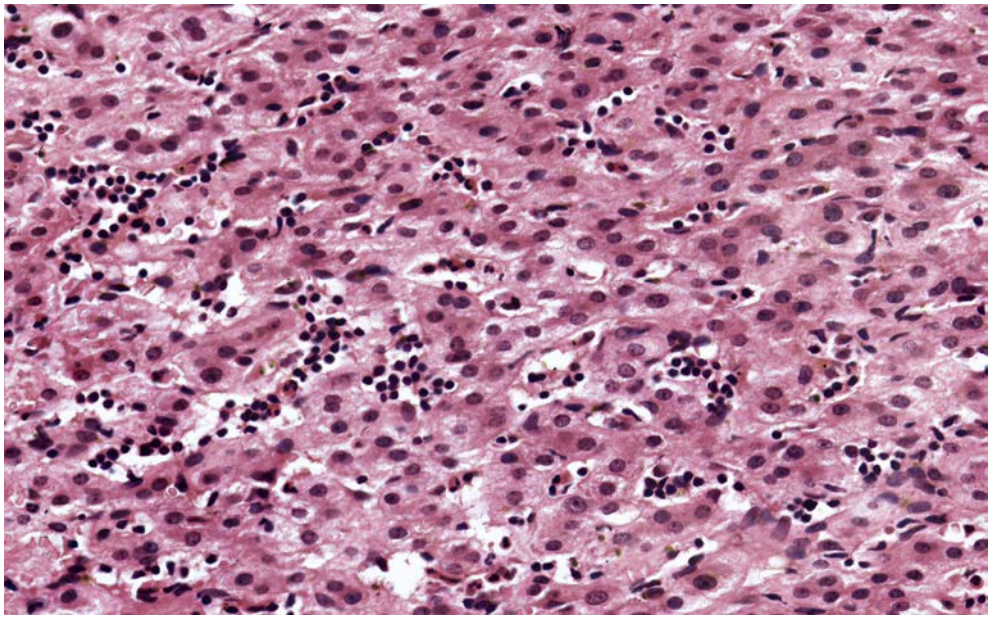

Fig. 15.14 Tropical splenomegaly syndrome (hyperreactive malarial splenomegaly). There are groups of lymphocytes in the sinusoids. Kupffer cells are enlarged. Hepatocytes appear normal. (Needle biopsy, H&E.)

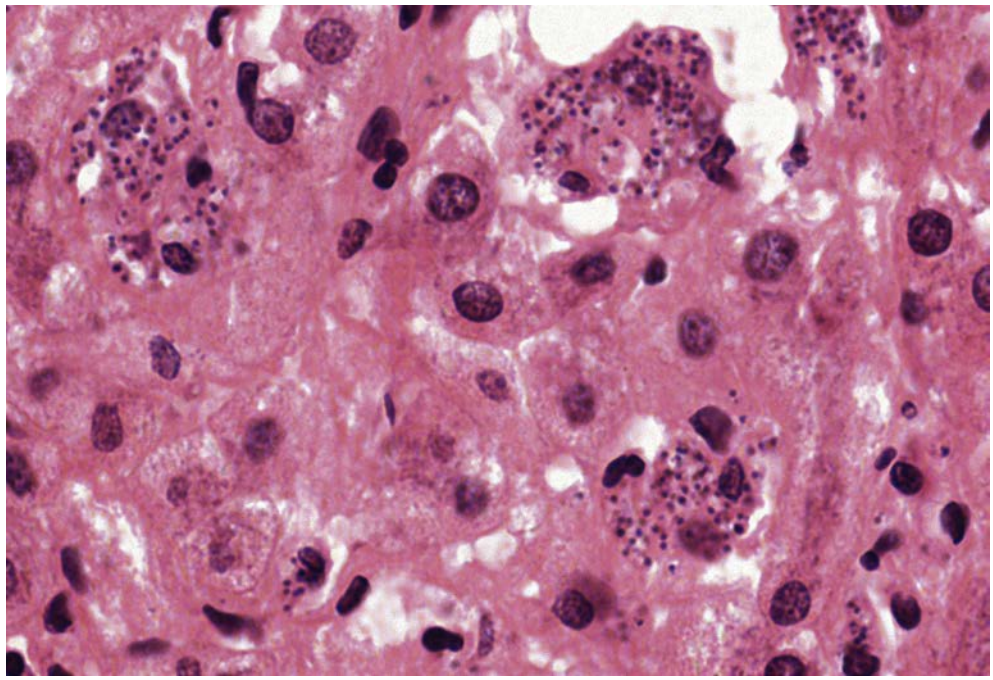

Fig. 15.15 Kala-azar. There are many Leishman–Donovan bodies within several hepatocytes, just large enough to give the cells a stippled appearance at this magnification. (Post-mortem liver, H&E.)

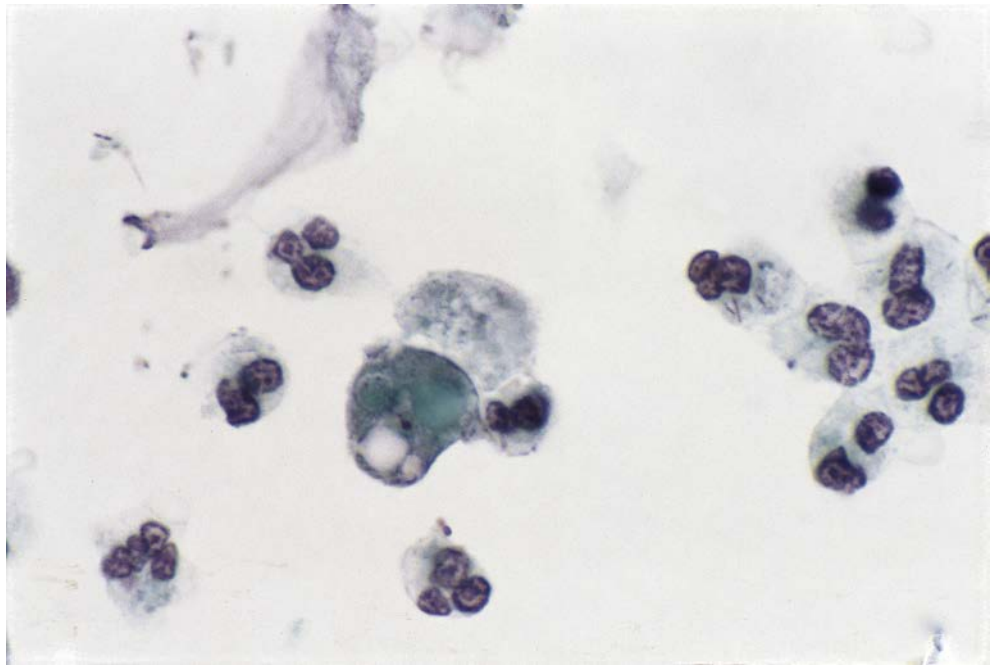

Fig. 15.16 Amoebic abscess. A trophozoite of *Entamoeba histolytica* is present in this fine-needle aspiration biopsy sample, with a round nucleus above several cytoplasmic glycogen vacuoles. Adjacent cells are neutrophilic leukocytes. (Papanicolaou.) (Illustration kindly provided by Dr Alastair Deery, London, United Kingdom.)

found in fibrotic and enlarged portal tracts. Medium-sized veins show intimal thickening which may be eccentric and polypoid, and in large veins there are thrombi and adult worms. In the course of portal scarring, isolated smooth-muscle cells may become separated from the portal-vein wall and entrapped in fibrous tissue, a helpful diagnostic feature.[217] Diffuse hyaline thickening and tortuosity of veins with surrounding fibrosis constitute 'clay pipestem fibrosis', in which hepatic artery branches and bile ducts are preserved (**Fig. 15.18**).[217]

Lobular changes are usually slight, but sinusoidal lining cells are prominent and there is an increase in fibre within the space of Disse.[218,219] Portal tract lymphocytic infiltrates and piecemeal necrosis are likely to reflect the presence of chronic hepatitis, as hepatitis B virus infection is increased in patients with hepatosplenic schistosomiasis,[220,221] as is hepatitis C.

Liver flukes

Invasion of the biliary tree by the trematodes *Clonorchis sinensis* (the Chinese liver fluke), *Opisthorchis viverrini* and *Opisthorchis felineus* is followed by proliferation of duct-like structures around the large bile ducts. The ductal epithelium may undergo goblet-cell metaplasia.[222] Smaller ducts are surrounded by an eosinophil-rich infiltrate. Complications include bile-duct obstruction, infection, portal fibrosis and hypertension, and bile-duct carcinoma.[223] Infestation may present several years after the patient has left an endemic area.[224]

The liver fluke *Fasciola hepatica* enters the liver from the peritoneal cavity and reaches the biliary tree some weeks later. White nodules are seen on the liver surface at the points of entry and may be mistaken for tumour. Migration tracks extend into the liver. Histologically, capsular and subcapsular lesions are composed of serpiginous areas of necrosis containing eosinophils and Charcot–Leyden crystals and bordered by palisaded histiocytes[224] (**Fig. 15.19**). Elsewhere in the liver, portal tracts are infiltrated with eosinophils. The biliary phase of the infestation is marked by cholangitis with rather less bile-duct hyperplasia than in *Clonorchis* or *Opisthorchis* infections, and both arterial and venous thrombosis. Features of bile-duct obstruction, periductal fibrosis and an ovum within a granuloma have also been reported.[225]

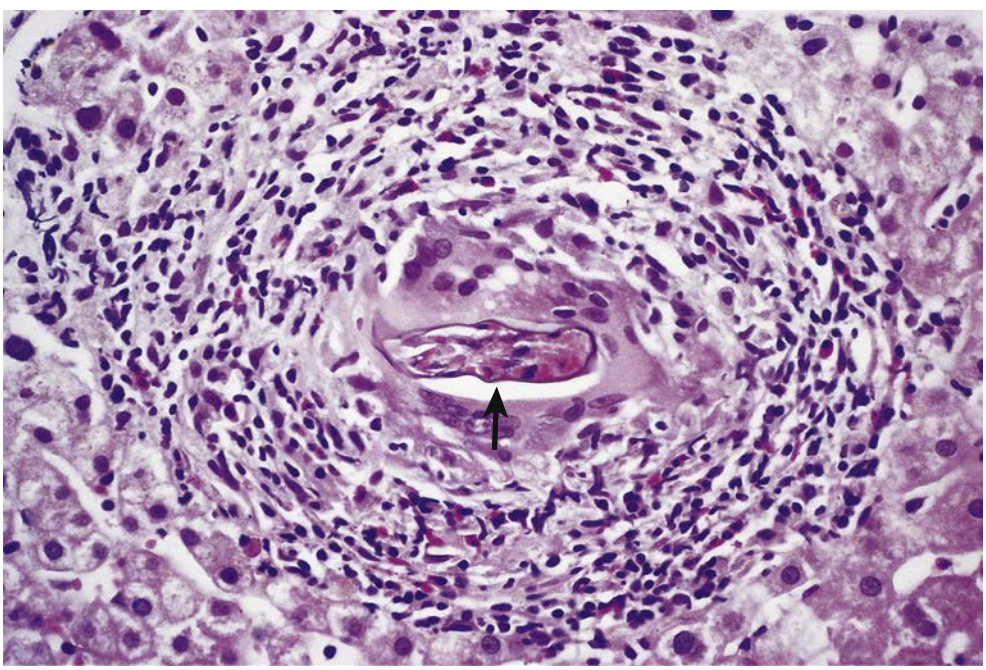

Fig. 15.17 Schistosomiasis. An ovum (arrow) surrounded by giant cells is seen at the centre of a granuloma. The infiltrate is rich in eosinophil leukocytes. (Needle biopsy, H&E.)

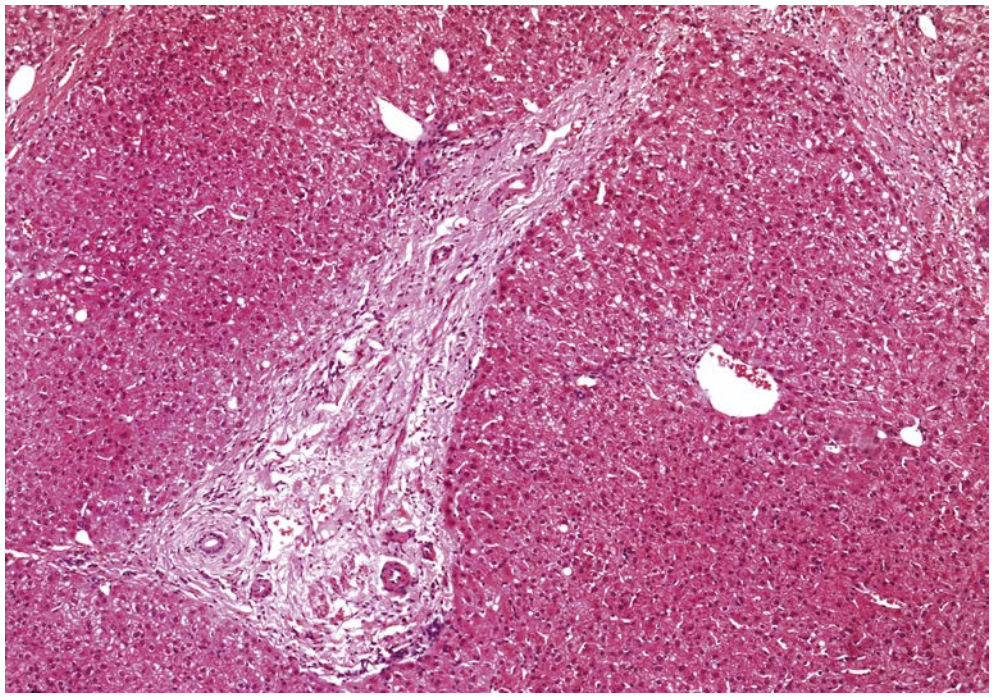

Fig. 15.18 Schistosomiasis. Serpiginous septa contain many small blood vessels in this example of 'pipestem' fibrosis. (Wedge biopsy, H&E.)

Ascariasis

Focal areas of necrosis with infiltration by eosinophils and neutrophils are seen in the migratory phase, when larvae travel to the lungs via the liver. Adult worms may enter the biliary tree from the duodenum, giving rise to bile-duct obstruction, cholangitis and abscess formation.[216]

Larval diseases

In several parasitic diseases with larval stages, including infestation by *Toxocara*, the larvae may reach the liver and give rise to eosinophil-rich abscesses or granulomas. Larvae are sometimes seen within these lesions.[12] White capsular and subcapsular liver nodules composed of mature fibrous tissue with calcification and few infiltrating cells surround larval remnants in long-standing disease due to *Toxocara* or to arthropod larvae.[226]

Gastrointestinal disorders and the liver

Patients with **coeliac disease** sometimes have elevated serum aminotransferases with nonspecific acinar or portal mononuclear inflammation, fatty liver or infrequently chronic hepatitis, cirrhosis and hepatocellular carcinoma.[227] Occasionally, coeliac disease is associated with primary biliary cholangitis or, rarely, with autoimmune hepatitis, primary sclerosing cholangitis or autoimmune cholangitis.[227,228] In **Whipple's disease,** characteristic foamy PAS-positive macrophages may be found in the liver,[229] and epithelioid-cell granulomas have been reported.[230] Granulomas, associated with a heavy infiltration by eosinophils, have also been described in **eosinophilic gastroenteritis**.[231] Gastrointestinal cancers (colorectal, pancreas, bile duct, small intestine) are sometimes associated with pyogenic liver abscesses.[232]

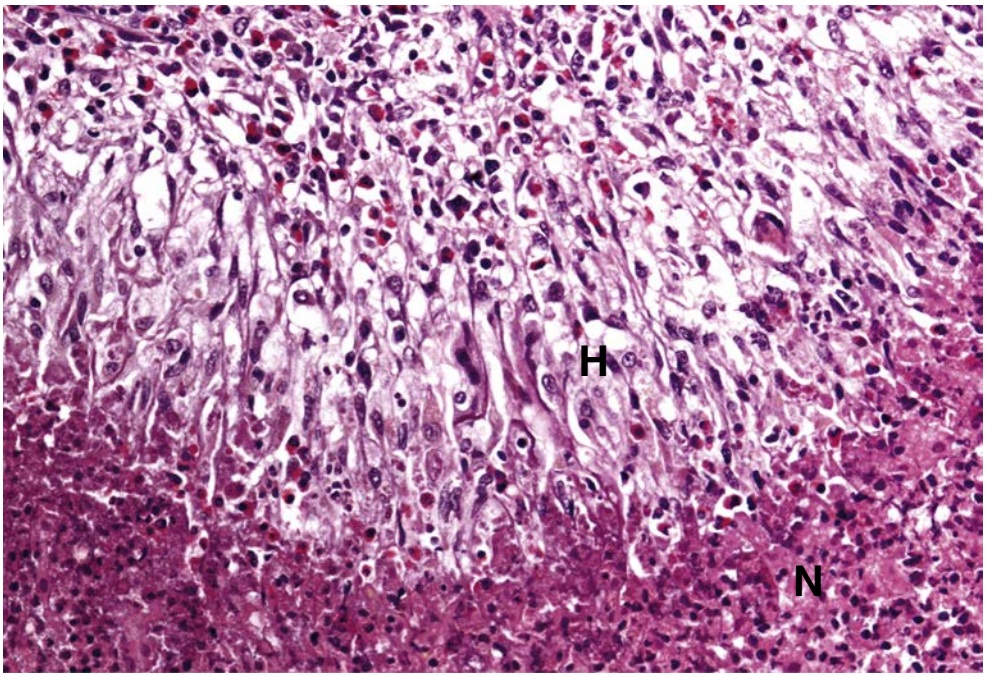

Fig. 15.19 Fascioliasis. Part of a nodule near the surface of the liver. A central area of necrosis (N) filled with leukocytes is bordered by palisaded histiocytes (H). Wedge biopsy, H&E.

Chronic inflammatory bowel disease

The spectrum of liver lesions is generally similar in ulcerative colitis and Crohn's disease. In Crohn's disease, serious hepatic complications such as sclerosing cholangitis are much less common, and there may be granulomas in the liver[233] or amyloid deposition.[234] Gallstones are more common in patients with Crohn's disease than in the general population. In both Crohn's disease and ulcerative colitis, malnutrition, anaemia and toxaemia can lead to steatosis.[235]

A minority of patients with ulcerative colitis have persistent abnormalities of liver function tests.[236] Careful examination including cholangiography shows that most of these patients have primary sclerosing cholangitis (**Ch. 5**). Bile-duct carcinoma, sometimes accompanied by diffuse dysplasia of biliary epithelium[237,238] (**see Fig. 5.18**), is increased in patients with ulcerative colitis, probably reflecting underlying sclerosing cholangitis.[239,240] Portal inflammatory lesions with or without periductal fibrosis in ulcerative colitis have occasioned use of the term 'pericholangitis'. However, patients with such portal inflammation have largely been shown to have typical primary sclerosing cholangitis[241] or its small-duct variant.[242] Furthermore, some examples of so-called pericholangitis probably represent a non-specific inflammatory response to the colitis. The term 'pericholangitis' should therefore be discarded.[242] While liver biopsies from patients with ulcerative colitis may show features of chronic hepatitis, this may be due to intercurrent viral hepatitis (e.g. following blood transfusion). However, it should be noted that interface hepatitis is also common in primary sclerosing cholangitis. From a practical point of view it therefore seems wise to consider the possibility of sclerosing cholangitis in all patients with ulcerative colitis and chronic liver disease.

Haematological disorders and the liver

One of the most common findings in liver biopsies from patients with haematological disorders is diffuse **Kupffer-cell siderosis**, usually reflecting prior transfusion (Ch. 14). In **reactive haemophagocytic syndrome**[243,244] diffuse Kupffer-cell hyperplasia with siderosis and phagocytosis of erythrocytes may be seen in patients with systemic infections, disseminated carcinoma, leukaemia and lymphoma. Kupffer-cell erythrophagocytosis in **macrophage activation syndrome** triggered by infection, malignancy or collagen vascular disease[245] is accompanied by portal and parenchymal CD8-positive T lymphocytes which may cause a clinical and histopathological hepatitis, the latter featuring prominent apoptotic bodies and, rarely, bile-duct damage or destruction.[246,247] Phagocytosed red blood cells are well demonstrated on chromotrope–aniline blue stain and the histiocytes stain much less intensely on diastase–PAS than those engaged in necrotising processes such as viral hepatitis. Hepatic involvement by leukaemias and lymphomas is discussed in Chapter 11, the effects of thrombosis and sickle-cell disease in Chapter 12 and graft-versus-host disease following bone marrow transplantation in Chapter 16.

Haemophilia

Hepatitis viruses are readily transmitted in blood products, and hepatitis is therefore common in patients with haemophilia.[248–251] Hepatitis C virus and possibly other putative non-A, non-E hepatitis viruses are the most important agents involved, but markers of infection with hepatitis B virus are also present in some patients. Liver histopathology is most often that of a mild chronic hepatitis[251]; cirrhosis is infrequent. The complications of infection by HIV-1 and AIDS have been seen in some haemophiliacs who received blood products contaminated with HIV-1 prior to mandated screening for the virus, which was initiated in the 1980s.

Extramedullary haemopoiesis

Haemopoiesis in the liver sinusoids is normal in fetal and neonatal life. In adults it is seen mainly in the myeloproliferative disorders and when tumours invade bone marrow. Foci of haemopoiesis may also be seen in the congested liver of patients with cardiac failure,[252] in massive hepatic necrosis,[253] in transplant livers with zone 3 necrosis[254] or in the rare situation of graft-versus-host disease in liver transplant recipients.[255] The sinusoids and spaces of Disse of the enlarged liver contain discrete clumps of haemopoietic cells (Fig. 15.20), and there are similar cells in portal tracts. Features that distinguish haemopoiesis from the infiltrates of leukaemias, infectious mononucleosis, other infections and the tropical splenomegaly syndrome are the variety of cells in the aggregates and the presence of recognisable marrow cells, such as normoblasts and eosinophil myelocytes. Megakaryocytes are commonly seen (Fig. 15.20) and are sometimes the only marrow elements found. Owing to the restraints of space, these cells are more elongated than in a section or smear of bone marrow. In the liver of neonates or stillborn infants with **Down syndrome**, megakaryocytes may be the predominant form of extramedullary haemopoiesis[256,257] and perisinusoidal fibrosis may also be present.[258] In approximately 5%–10% of Down syndrome patients, transient abnormal myelopoiesis (TAM) is associated with intra-sinusoidal myeloid blasts with features of megakaryocytic lineage (Fig. 15.21A). This usually resolves within 3 months. TAM is a result of the synergistic effects of trisomy 21 and *GATA1* mutation.[259] However, in about 20% there is risk of developing extensive sinusoidal fibrosis (Fig. 15.21B) and liver failure.[259,260]

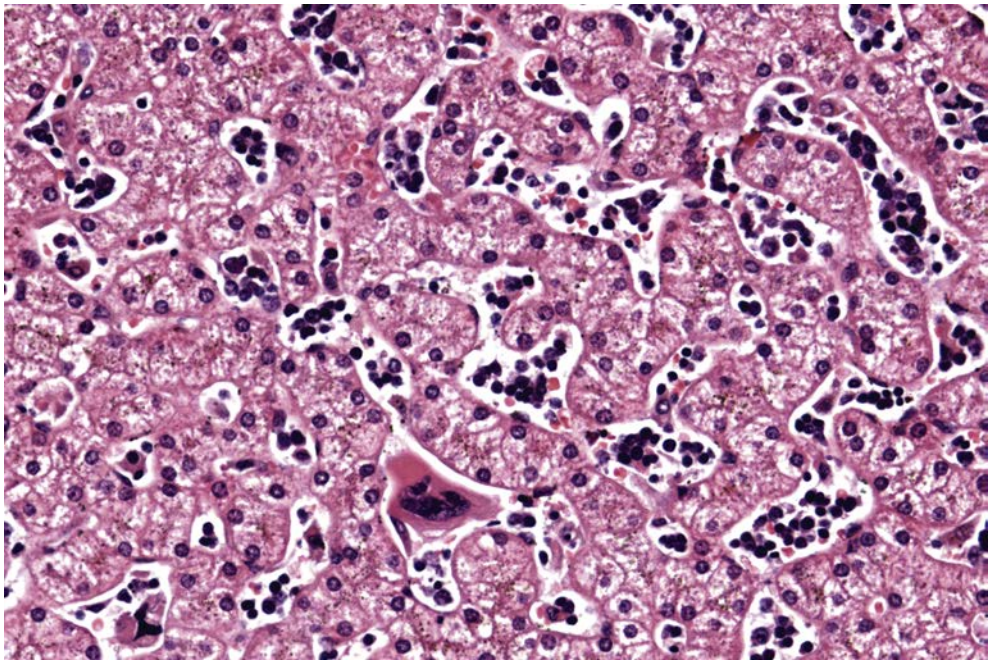

Fig. 15.20 Extramedullary haemopoiesis. Clumps of haemopoietic cells are seen in the sinusoids, including a megakaryocyte at the bottom of the field. (Needle biopsy, H&E.)

The liver in rheumatoid, immune-complex and collagen diseases

Liver pathology is uncommon in this group of diseases, but, when present, is most often **steatosis**.[261] **Nodular regenerative hyperplasia** is also seen in many connective tissue diseases.[262,263] The presence of multisystem disordered immunity in these conditions may be reflected in some cases by associated immune damage to bile ducts (**primary biliary cholangitis**) or in the form of hepatic vasculitis.[263,264]

Polyarteritis nodosa in small hepatic arteries can lead to infarction. Immune complexes containing hepatitis B surface antigen are sometimes demonstrable in vessel walls in this disease.[265] Both hepatitis B and C virus antigen–antibody complexes have been implicated in the pathogenesis of **essential (type II) mixed cryoglobulinaemia**.[266–269] In the **polymyalgia rheumatica-giant-cell arteritis syndrome**, the liver may contain granulomas.[270,271] Other findings reported include fatty change, venous congestion, non-specific hepatitis and prominent stellate cells.[272,273]

Patients with **rheumatoid arthritis** often have abnormal liver function tests, but liver biopsy more often shows non-specific changes or normal liver than definitive liver disease.[274–276] Amyloidosis or necrotising arteritis may be found, and rheumatoid nodules in the liver such as those typically found in subcutaneous tissue have been reported.[277] In **Felty's syndrome**[278] (rheumatoid arthritis, leucopaenia and splenomegaly), the nodular lesions may be attributable to arteritis involving small intrahepatic vessels.[279]

Scleroderma and the **CRST syndrome** (calcinosis, Raynaud's phenomenon, sclerodactyly and telangiectasia) may be associated with primary biliary cholangitis.[280,281] Giant, dense mitochondria on electron microscopy, with normal liver or non-specific changes on light microscopy, have been described in patients with **systemic sclerosis**.[282]

Most patients with SLE do not have significant liver pathology but chronic hepatitis, cirrhosis and hepatic granulomas have been reported.[283,284] Abnormal liver function tests

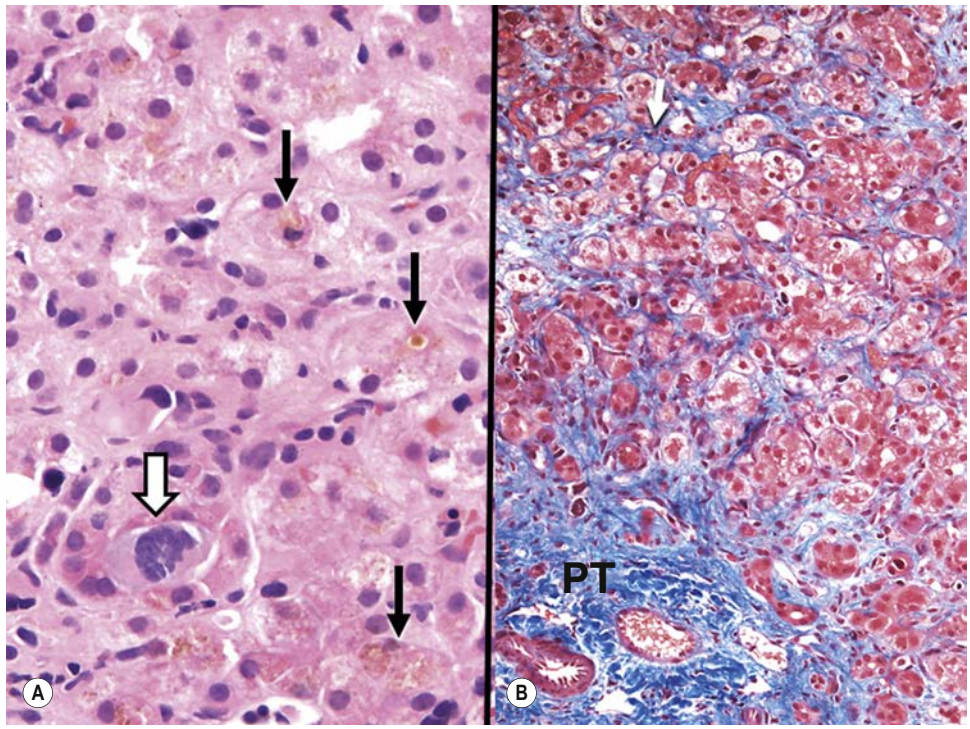

Fig. 15.21 Down syndrome with transient abnormal myelopoiesis. A: Atypical megakaryocytic forms (white arrow) are seen within the hepatic sinusoids. Hepatocellular and bile canalicular cholestasis is also apparent (black arrows). **B:** Down syndrome patients with transient abnormal myelopoiesis may develop fibrosis and liver failure. Note the extensive pericellular/perisinusoidal, as well as the periportal, fibrosis present in this case (PT = portal tract). (Needle biopsy; **A:** H&E; **B:** Masson trichrome stain.)

may be present without serious lesions.[285,286] Other changes described in SLE include steatosis,[287] cholestasis, nodular regenerative hyperplasia and necrotising arteritis involving arteries of 100–400 μm diameter.[284] An unusual case of **malacoplakia** involving the liver in a steroid-treated patient with SLE and Gram-negative bacterial infection showed aggregates of histiocytes with typical Michaelis–Gutmann bodies.[288] Although there appears to be no close relationship between systemic lupus and autoimmune (lupoid) hepatitis, SLE with autoimmune hepatitis or with primary biliary cirrhosis may coexist.[287] The case of a patient with chronic hepatitis and **mixed connective tissue disease** has been reported.[289]

Amyloidosis and light-chain deposition

The liver is commonly involved in systemic amyloidosis. 'Primary' (AL) and reactive (AA) amyloidosis cannot definitively be distinguished by the pattern of liver involvement,[290,291] although sinusoidal deposition in AL and vascular involvement in AA are consistent patterns reported in several studies.[290,292] Histological distinction is made by the resistance of AL amyloid to potassium permanganate before Congo red staining[293] and by immunohistochemistry for immunoglobulin light chains, AA protein, transthyretin and other proteins.[291,294,295] In most patients the amyloid is deposited in portal arteries (**Figs 15.22 and 15.23**) or diffusely in the perisinusoidal space of Disse (**Fig. 15.24**). The two patterns are often combined. The perisinusoidal deposits compress both the sinusoids and the liver-cell plates, occasionally leading to portal hypertension or to cholestasis.[296–298] Rarely, AL or AA amyloid may be in the form of globular deposits[299,300] in the space of Disse (**Fig. 15.25**),

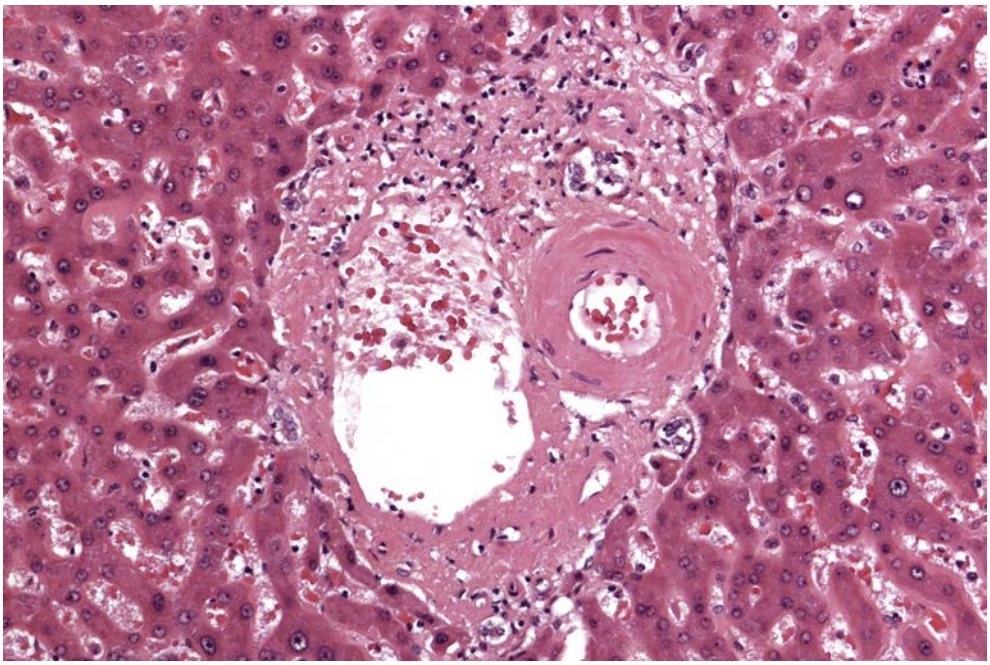

Fig. 15.22 Amyloidosis. The artery at right within this portal tract is thickened by amyloid deposit. (Needle biopsy, H&E.)

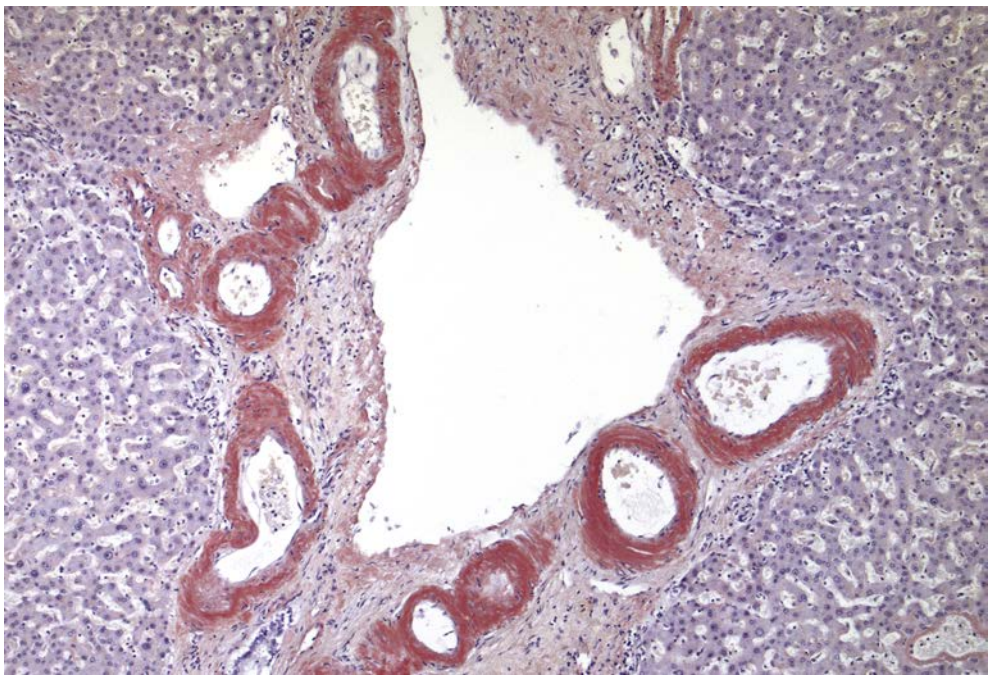

Fig. 15.23 Amyloidosis. Arterial amyloid deposits in this large-calibre portal tract are highlighted by Congo red staining. (Wedge biopsy, Congo red.)

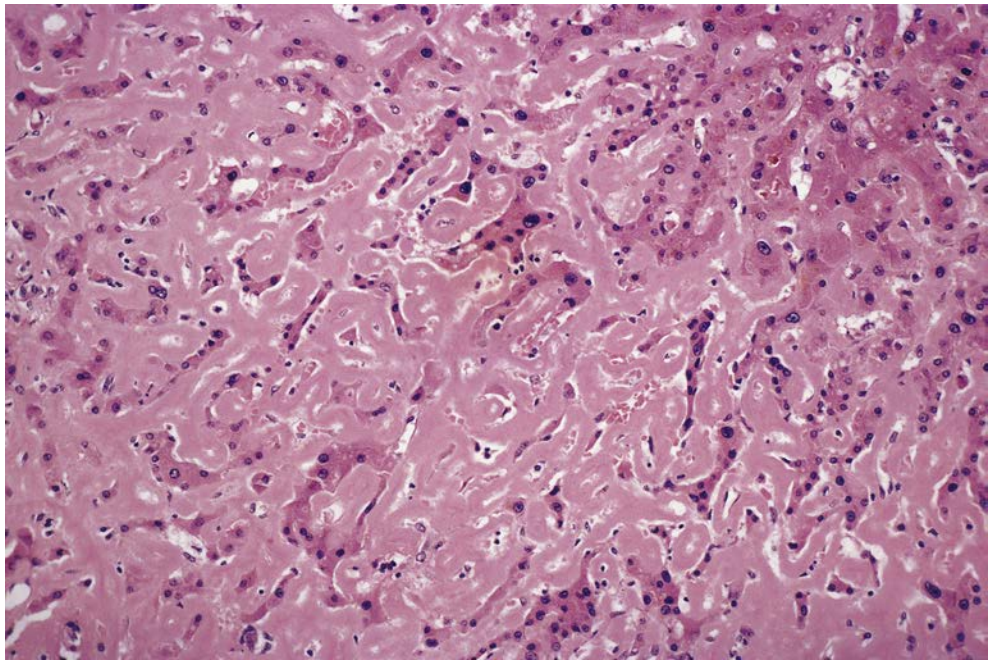

Fig. 15.24 Amyloidosis. Amyloid has been laid down in the space of Disse. Liver-cell plates have undergone atrophy, and sinusoids are narrowed. Cholestasis, an unusual complication of hepatic amyloidosis, is seen at upper right. (Explant liver, H&E.)

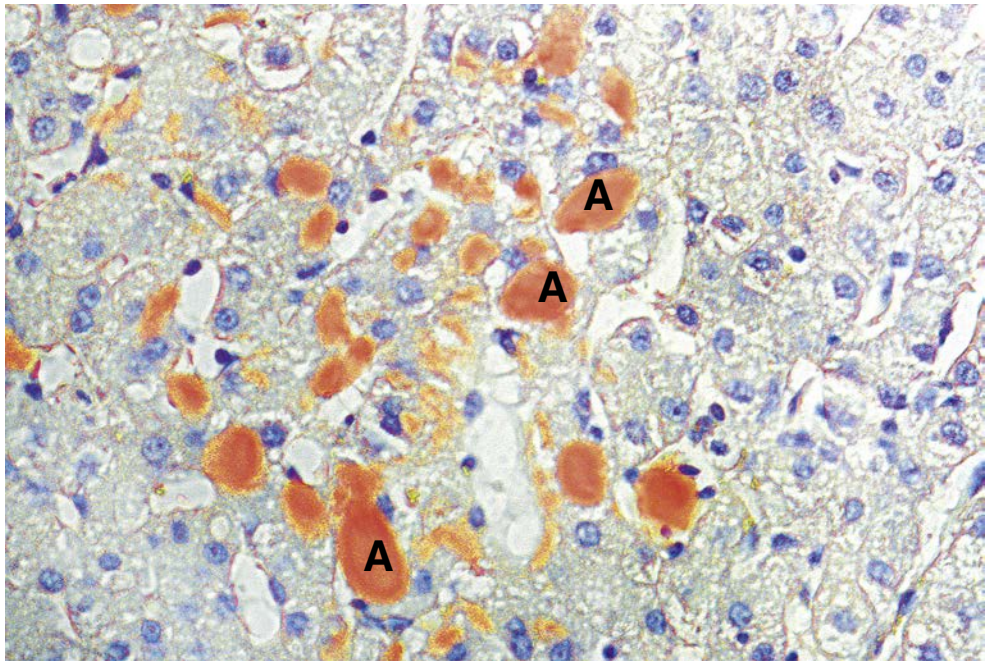

Fig. 15.25 Globular amyloid. There are rounded deposits of amyloid (A) in the space of Disse. (Needle biopsy, Congo red.)

within or surrounding portal arteries and veins or terminal venules, or within hepatocytes. While the constituent protein within the globular amyloid is infrequently AL or AA type (4% in one study[300]), most globular amyloid is composed of ALECT2 (amyloid leukocyte chemotactic factor-associated) protein.[300] Apolipoproteins and fibrinogen A are other uncommon types of non-globular hepatic amyloid.[300]

Amorphous perisinusoidal and portal deposits that are somewhat like amyloid are seen when the liver is involved in light-chain deposit disease.[301] Immunoglobulin light chains, usually kappa, can be identified immunochemically. The characteristic green birefringence of amyloid after Congo red staining is absent. Occasionally, amyloid and light-chain deposits are found in the same patient.[302,303]

The liver in the porphyrias

Liver lesions are found in **porphyria cutanea tarda** (PCT) and **protoporphyria.**[304] Red porphyrin fluorescence can be demonstrated in both diseases using unfixed liver tissue.

In **PCT**, hepatocytes contain needle-shaped birefringent porphyrin crystals, sufficiently water-soluble to make their demonstration difficult or impossible in routinely prepared paraffin sections. They can be seen in unstained paraffin sections, with a ferric ferricyanide reduction stain[305] and in H&E-stained sections prepared with minimal exposure to water.[306] Fatty change and hepatocellular siderosis are common[307] and intra-acinar clumps of iron- and ceroid-containing macrophages, fat droplets and inflammatory cells may also be present.[308] The role of *HFE* mutations in PCT varies geographically.[309–311] Alcohol contributes to the pathogenesis of PCT, and biopsies should be critically examined for alcohol-related injury. More importantly, **chronic hepatitis** and **cirrhosis** are common in patients with PCT, and the majority have hepatitis C virus infection.[312–315] The prevalence of chronic hepatitis C in this population is approximately 50%,[316,317] which may also explain the development of **hepatocellular carcinoma**.[304]

In **protoporphyria** (erythropoietic or erythrohepatic protoporphyria), dense, dark brown deposits of poorly soluble protoporphyrin accumulate in the liver (**Fig. 15.26**). These give a diagnostic red birefringence under polarised light, with a characteristic dark Maltese cross centrally.[318] There may be serious liver damage, with cholestasis, perisinusoidal and perivenular fibrosis and cirrhosis developing in some patients.[319,320]

Non-specific reactive changes

A variety of changes including portal and lobular inflammation, fat accumulation and Kupffer-cell hypertrophy are seen in extrahepatic conditions, especially febrile, inflammatory or widespread neoplastic diseases. Focal hepatocellular necroses may be found in the parenchyma. The distinction of these reactive changes from a mild form of chronic hepatitis or from residual acute hepatitis requires clinical information. The latter may sometimes be suspected from a predominantly perivenular location of inflammation and liver-cell loss. Reactive changes near space-occupying lesions such as metastatic tumours are discussed in **Chapter 1** (**see Fig. 1.5**).

The liver in pregnancy

In normal pregnancy there are no specific light-microscopic findings in the liver. Electron-microscopic changes reported in late pregnancy include giant mitochondria with paracrystalline inclusions, increase in the number of peroxisomes and proliferation of smooth endoplasmic reticulum.[321]

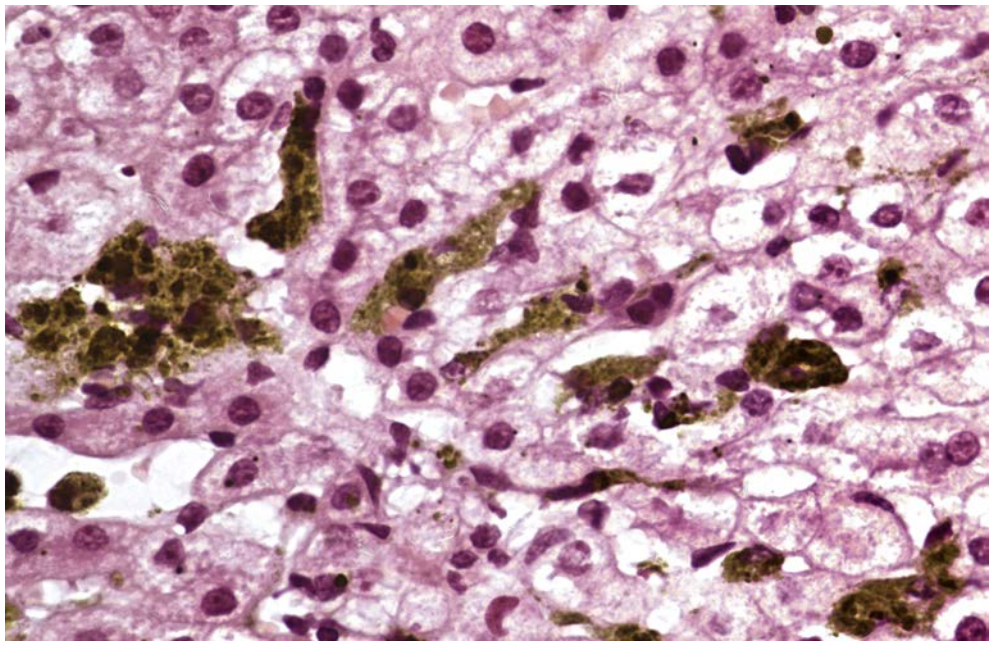

Fig. 15.26 Erythropoietic protoporphyria. Dense brown protoporphyrin deposits are seen within sinusoids. (Needle biopsy, H&E.)

Liver disease in pregnancy is rare and falls into three categories[322]:

1 **Liver disease unique to pregnancy.** The four conditions included in this category[323–325] are **acute fatty liver of pregnancy** (AFLP), **pre-eclampsia/eclampsia**, the **HELLP syndrome** (haemolysis, elevated liver enzymes and low platelets) and **intrahepatic cholestasis of pregnancy** (ICP).
2 **Intercurrent liver disease during pregnancy.** Viral hepatitis and cholelithiasis are examples. Hepatocellular carcinoma, including the fibrolamellar variety,[326] occurs rarely. **Viral hepatitis** is the most common form of liver disease encountered in pregnancy.
3 **Pre-existing liver disease in the pregnant patient.** Chronic hepatitis B viral infection and autoimmune hepatitis with or without cirrhosis are examples.

Jaundice and elevated serum aminotransferases are important aspects of the clinical presentation of liver disease in pregnancy. The following discussion is limited to those diseases unique to pregnancy.

Acute fatty liver of pregnancy

This uncommon and serious complication of pregnancy develops in the last weeks of gestation[327–329] and in certain cases is caused by gene mutations affecting mitochondrial fatty acid oxidation enzymes.[330,331] Steatosis involves the greater part of each acinus, usually leaving a thin and incomplete rim of normal hepatocytes around the portal tracts.[332,333] The fat is mainly in the form of fine droplets, as in Reye's syndrome and other examples of microvesicular steatosis.[333,334] Large fat vacuoles of the kind seen in alcoholic liver disease are scanty, and the cause of the hepatocellular swelling and pallor may not be readily apparent on examination of paraffin sections (**Fig. 15.27**). PAS and trichrome stains are sometimes more helpful than H&E for identifying the small fat vacuoles. Fat staining of frozen sections makes the diagnosis clear, and a piece of the biopsy specimen should

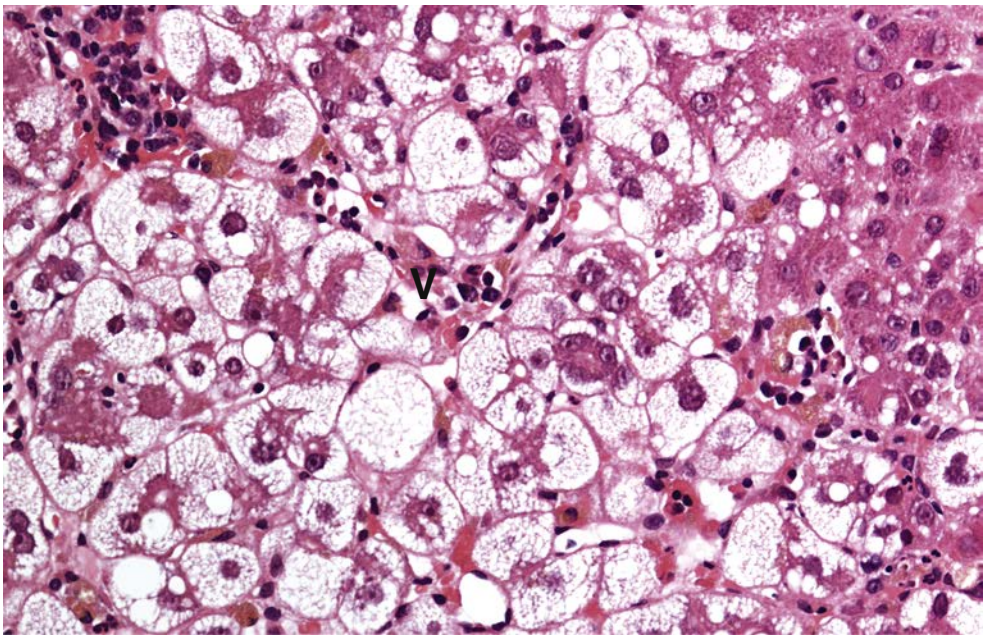

Fig. 15.27 Acute fatty liver of pregnancy. Swollen pale-staining hepatocytes are seen around a terminal hepatic venule (V). The edge of a portal tract with a mild lymphocytic infiltrate is seen at the upper left corner. No large fat vacuoles are seen. (Postmortem liver, H&E.)

therefore be kept for frozen sectioning in patients with unexplained jaundice in late pregnancy. Inflammatory cells, mostly lymphocytes, are prominent in some examples and may lead to confusion with acute viral hepatitis,[335] in which microvesicular steatosis is not seen. In more severe examples of AFLP, there is loss of hepatocytes leading to approximation of portal tracts. Fibrin deposits are occasionally demonstrable in hepatic sinusoids. One series showed cholestasis, extramedullary haemopoiesis and giant mitochondria[333] in some patients. With rare exceptions,[336] AFLP does not recur in subsequent pregnancies.

Pre-eclampsia/eclampsia

Hypertension, proteinuria, peripheral oedema and occasional coagulation abnormalities in pregnancy constitute pre-eclampsia, or eclampsia if convulsions and hyperreflexia are also present. Liver involvement is unusual, but may be manifested by elevated levels of serum aminotransferases and/or alkaline phosphatase. The incidence of pre-eclampsia is increased in patients with acute fatty liver.[335] The liver in pre-eclampsia shows fibrin thrombi in portal vessels and periportal sinusoids (**Fig. 15.28**) associated with necrosis and haemorrhage in more severe cases.[337,338] The identity of the fibrin can be established by phosphotungstic acid–haematoxylin staining or immunofluorescence.[339] These changes are not seen in all patients. Infarction, haematoma and rupture of the liver[340] are complications.

HELLP syndrome

This syndrome is exceedingly uncommon in pregnancy[327,341–344] and is seen in 20% of patients with severe pre-eclampsia.[323] Liver biopsy changes range from non-specific portal inflammation and glycogenated hepatocyte nuclei[327,342–344] to the periportal fibrin and necrosis seen in pre-eclampsia.[323] One study demonstrated a relationship between maternal AFLP and HELLP syndrome and a mitochondrial fatty acid γ-oxidation disorder with 3-hydroxyacyl-CoA dehydrogenase deficiency in their offspring.[345a] Two of the children in the study had severe fatty change, necrosis and early nodules at autopsy. Rarely, hepatic

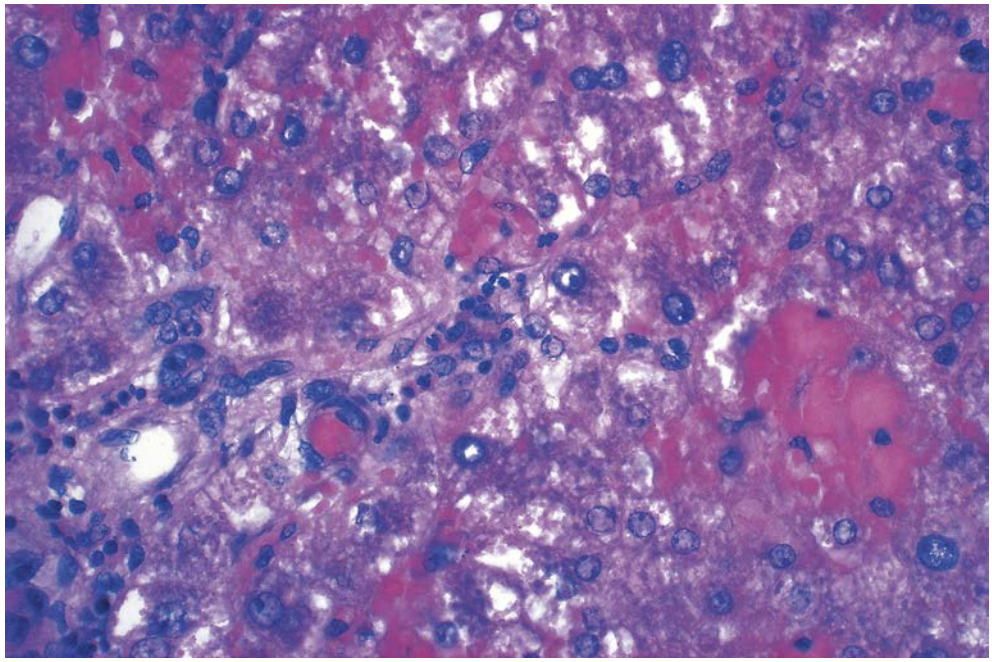

Fig. 15.28 Pre-eclampsia/eclampsia. Fibrin is seen within the periportal sinusoids at lower right. (Needle biopsy, H&E.)

infarct, rupture or haematoma may develop.[345b] Hepatic changes in HELLP syndrome may occur due to release of placental factors that produce sinusoidal endothelial toxicity resembling that seen in sinusoidal obstruction syndrome (SOS) (see Ch. 12).[345c]

Intrahepatic cholestasis of pregnancy

Pruritus, with or without cholestatic jaundice, may develop in late pregnancy and recur in subsequent pregnancies. It regresses after delivery. Liver biopsy shows little apart from canalicular cholestasis, most severe in perivenular areas.[337] Minor hepatocellular changes and inflammation are attributable to the cholestasis itself. Portal inflammation is absent or mild. No histological abnormalities are detectable between pregnancies, but the jaundice has been shown to return on administration of oral contraceptives.[346] Concomitant ICP and AFLP have been reported.[347] Some 15% of cases are accounted for by mutations in the multidrug resistance 3 (*MDR3*) bile transport gene.[348–351] Gallstone disease and hepatitis C virus infection are also associated with ICP, both before and after pregnancy.[352]

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CHAPTER

16 The Liver in Organ Transplantation

Introduction

The pathologist is often asked to examine liver biopsies obtained to evaluate liver dysfunction in transplant patients, including recipients of liver, renal and bone marrow grafts. For liver transplantation, liver biopsy remains the diagnostic 'gold standard' when jaundice and allograft dysfunction develop, because biochemical tests do not adequately discriminate between rejection and other conditions that may develop in the allograft.[1] Moreover, even when serum liver function tests are normal, histological abnormalities (including rejection lesions) may be present.[2,3] At the time of harvesting or engraftment, the donor liver may also require assessment, sometimes by frozen section, for lesions that may determine whether or not the graft can be used, and that can affect the postoperative course and appearance of subsequent posttransplantation biopsies. This chapter reviews the histopathological features of liver transplant rejection and other conditions affecting the allograft. The concluding sections discuss liver disease in recipients of renal and bone marrow transplants.

Liver transplantation

Assessment of the donor liver

The source of the donor allograft is an important consideration when evaluating liver biopsies obtained prior to transplantation, after graft revascularisation or at later times. Biopsies from potential living donors in apparent good health may disclose conditions such as steatohepatitis or primary biliary cholangitis (PBC), which disqualifies their candidacy.[4] Living donor left- or right-lobe grafts, if small in relation to the recipient's size, may show cholestasis or congestion associated with ascites, prolonged coagulation parameters and impaired metabolic function immediately after transplantation (**small-for-size syndrome**[5]) because of problems in venous drainage[6] and portal hyperperfusion.[5] Postoperative portal tract biliary obstructive changes may also be present due to ischaemia or mechanical obstruction of the large bile ducts associated with this type of procedure.[7] Cadaveric livers are subject to preservation (ischaemia/reperfusion) injury[8] after harvesting and transport to the site of surgery, which in early baseline biopsies is evident as variable degrees of perivenular necrosis, liver-cell ballooning and/or apoptosis. The increased risk of postoperative biliary strictures and bile leaks with 'donation after cardiac death' grafts[9] should be kept in mind if cholestasis and features of biliary obstruction are seen in a post-transplantation biopsy. Liver transplantation where 'extended donor criteria' are invoked[10] (e.g. advancing age, donation after cardiac death, hepatitis virus infection, others) often requires morphologic assessment prior to the actual transplantation and allograft revascularisation. Donors with known chronic hepatitis B or C benefit from baseline biopsy grading and staging as a source for comparison in the event that later biopsies are obtained. With the advent of direct-acting antiviral agents (DAAs) that effectively treat hepatitis C virus (HCV), there is increased interest in

using HCV-seropositive deceased donor livers for transplantation to HCV-seronegative recipients (so-called HCV mismatch transplantation).[11–13] In the United States, increasing deaths from opioid abuse have increased an available pool of HCV-positive livers (and other organs) for transplantation.[12,13] The operative principle in 'HCV mismatch transplantation' is that antiviral therapy after organ transplantation will effect cure and serologic sustained viral response (SVR). Liver biopsy is likely to remain important in this setting, because antiviral-treated liver transplant recipients with SVR often continue to demonstrate chronic inflammation and fibrosis on liver biopsy and, in certain cases, may also still harbour HCV RNA in liver tissue.[14]

Allograft biopsies obtained soon after transplantation sometimes disclose an unsuspected condition in the donor, such as α_1-antitrypsin deficiency, iron overload or amyloidosis.[15] Certain known genetic, haematological or immunological disorders in potential donors may constitute contraindications.[16]

Frozen section of potential donor livers may be requested to exclude pre-existing disease. Pathologists providing frozen-section coverage should be aware of three common reasons why frozen sections are requested: (1) *to determine whether steatosis is present*, and its degree; (2) *to exclude changes of chronic hepatitis* if the donor is known to be positive for antibodies to hepatitis B core antigen but negative for surface antigen (i.e. possible occult hepatitis B), or is positive for HCV; and (3) *to evaluate a mass* found in the donor liver. Concern about a substantially **fatty liver** is based on the increased incidence of **primary graft dysfunction** or **non-function** when this is present.[17] The transplant surgeon may be concerned about significant steatosis when the donor liver appears yellow, has rounded edges and shows no surface capsular scratch marks (foci of capsular collagen rupture and disruption, thought to be a procurement phenomenon due to proximity to ice crystals).[18,19] The degree of macrovesicular (large droplet) fatty change should be categorised according to the percentage of parenchymal involvement as absent, mild (<30%), moderate (30%–60%) or marked (>60%).[20,21] Transplant surgeons have considered the last category unsuitable for use because of the high risk of primary dysfunction or non-function associated with severe steatosis.[17,21] Microvesicular (small-droplet) fat is held not to be a contraindication, however,[22] but if it is substantial, it should also be graded and merits discussion with the transplant team because it may delay return of hepatic function and clinical recovery.[23] Diffuse portal mononuclear inflammatory cell infiltrates in donors with markers of hepatitis B or C viral infection support the presence of **chronic hepatitis**. The significance of this finding needs to be considered by the transplant team. With regard to **mass lesions** in the donor liver, demonstration of a malignant or metastatic tumour is an obvious contraindication to its use. However, the features of benign lesions such as focal nodular hyperplasia (**Ch. 11**) are important to recognise because they are often encountered in this setting.

Box 16.1 Pathological considerations in the transplant liver

Graft rejection
Humoral (hyperacute)
Acute (cellular)
Chronic (ductopenic)
Functional cholestasis
Preservation injury
Bile-duct obstruction
Thrombosis of hepatic artery or portal vein
Infections and sepsis
Drug toxicity
Recurrence of original disease
Neoplastic disease
Posttransplant lymphoproliferative disease
Hepatocellular carcinoma

The liver allograft biopsy: general considerations

Needle liver biopsies are obtained as part of a liver transplantation protocol or because of clinical deterioration.[24] Discussion with the clinical team and careful review of pertinent radiographic, biochemical and microbiological findings are critical to biopsy interpretation and institution of appropriate therapy. Serial biopsies may be necessary to resolve difficult diagnostic problems.

There are many causes of allograft injury in addition to rejection (**Box 16.1**), and these should be considered in the context of the time elapsed since transplantation[25] (**Fig. 16.1**). For several weeks following transplantation, **functional cholestasis** may be present and must, if possible, be distinguished from the cholestasis of acute

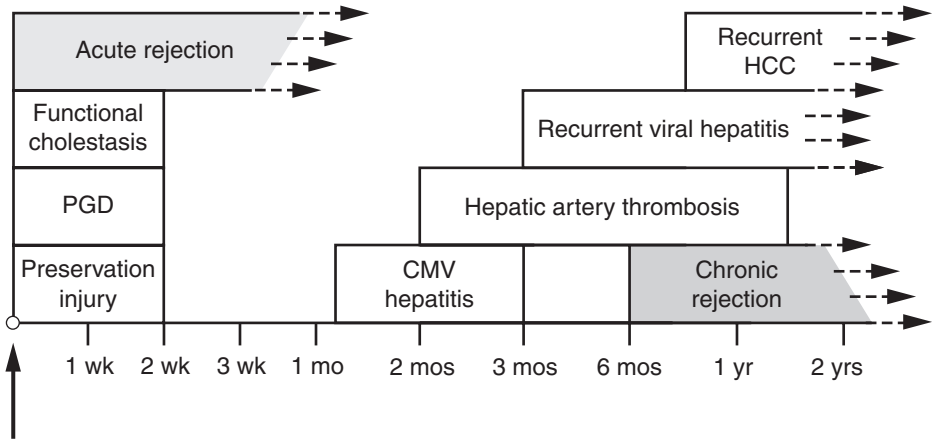

Fig. 16.1 Timeline of pathological lesions after liver transplantation. Common posttransplantation problems are shown correlated with the approximate time frame in which they develop. Hatched arrows indicate the potential for the condition to develop at a later time. CMV, Cytomegalovirus; HCC, hepatocellular carcinoma; PGD, primary graft dysfunction, Tx, transplantation.

rejection, bile-duct obstruction, hepatitis, drug toxicity and sepsis. Bile is present within hepatocytes and canaliculi. This impairment of bile flow can be explained by exposure of the donor liver to cold ischaemia and reperfusion injury (preservation injury) with resultant damage to liver-cell organelles.[26] Liver-cell death due to preservation injury actually shows features of both necrosis and apoptosis (necrapoptosis).[27] Early postoperative cholestasis may also be due to a 'small-for-size' graft.[28] Cholestasis may be accompanied by **hepatocellular ballooning** in perivenular regions (**Fig. 16.2**) or in a diffuse distribution.[29,30] In the absence of frank perivenular necrosis, ballooning does not confer an unfavourable prognosis.[29] Hypoperfusion liver damage in the perioperative period may result in necrosis in periportal or perivenular regions and sometimes an irregular subcapsular band of infarction.[31] If the donor liver is fatty, rupture of hepatocytes affected by preservation injury may rarely cause sinusoidal engorgement by lipid vacuoles (**lipopeliosis**)[32] (**Fig. 16.3**).

In evaluating posttransplant biopsies, special attention should be paid to the portal tracts, the major sites of rejection lesions. The type of cellular infiltrate, the bile ducts, portal-vein branches and hepatic arterioles are examined to distinguish rejection from other conditions with portal tract pathology, particularly **bile-duct obstruction**, **recurrent viral hepatitis**, **drug toxicity** and immunosuppression-related **lymphoproliferative disease** (see 'Differential diagnosis in transplant biopsies' section, later). The perivenular region also requires inspection for possible preservation injury, cholestasis or inflammation, and for necroinflammation which may accompany portal tract lesions in more severe cases of acute rejection.[33] The lobular parenchyma shows few alterations in rejection apart from cholestasis and the occasional apoptotic bodies and scattered liver-cell mitoses which develop as the allograft equilibrates to the appropriate size for the recipient. As a result, in cases where confusion arises in the interpretation of portal changes, it is important to evaluate the lobular parenchyma carefully for evidence of intercurrent diseases such as viral or drug hepatitis. *The pathologist should always bear in mind that a given biopsy may show superimposed features attributable to several different posttransplantation complications.*

Graft rejection

The histopathological lesions of liver allograft rejection have been well characterised[34–38] and are classified as **humoral rejection**, **acute (cellular) rejection** and **chronic (ductopenic) rejection**, as recommended by an international working party which met in 1994.[33] Acute and chronic rejection are the most common forms seen in clinical practice.

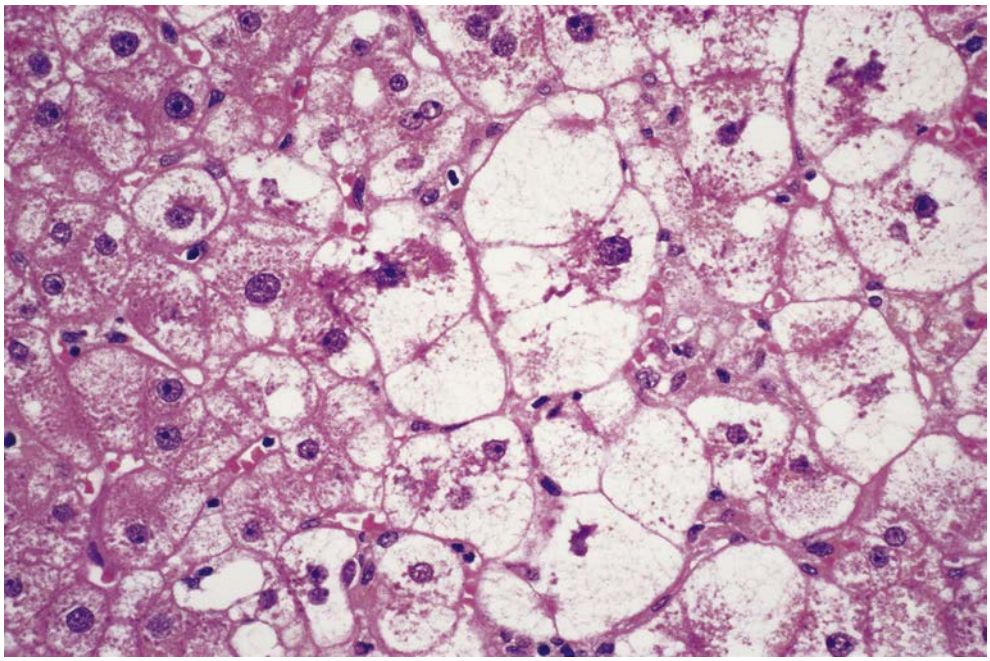

Fig. 16.2 Liver-cell ballooning after transplantation. A liver biopsy obtained in week 2 following transplant shows ballooning of hepatocytes in a perivenular area. Intracellular cholestasis is visible. (Needle biopsy, H&E.)

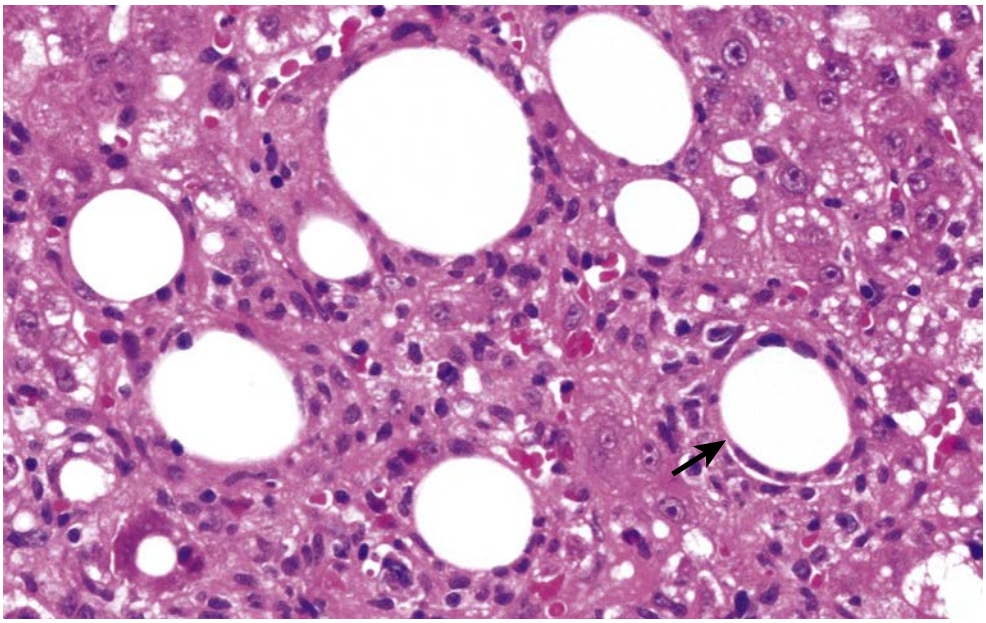

Fig. 16.3 Lipopeliosis. The enlarged empty spaces in this perivenular region represent ruptured and coalescent lipid vacuoles within sinusoids. This developed due to necrosis and rupture of steatotic hepatocytes following allograft preservation injury. A Kupffer-cell foreign-body reaction engulfing the lipid is present (arrow). (Needle biopsy, H&E.)

Antibody-mediated (humoral) rejection

Antibody-mediated rejection (AMR) is rare after liver transplantation and has been best studied in recipients of ABO-incompatible allografts. Microvascular damage evolves over the first few hours after transplantation, consisting of sinusoidal infiltrates of neutrophilic leukocytes, fibrin and red blood cells associated with focal haemorrhages. This progresses to portal and periportal oedema with coagulative and haemorrhagic necrosis and a ductular reaction over the next few days[39] (Fig. 16.4). Immunofluorescent studies show linear

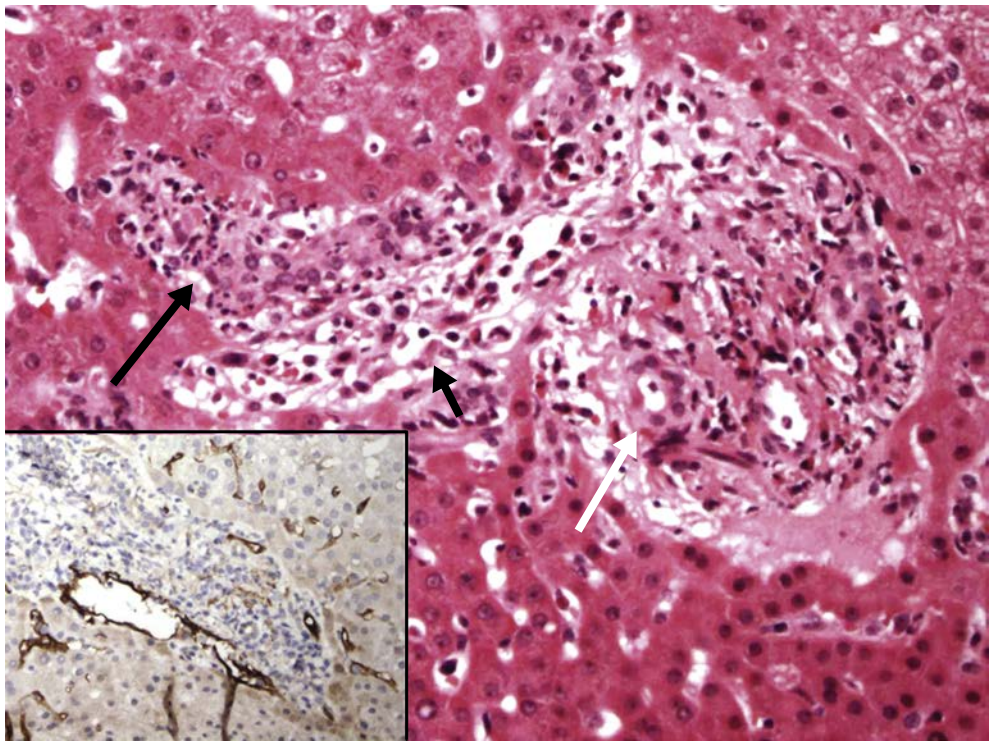

Fig. 16.4 Antibody-mediated rejection after ABO-incompatible liver transplantation. There is mild portal tract oedema with an early periportal ductular reaction and increased neutrophils (long arrow) in this 1-week postoperative allograft biopsy. The portal-vein branch (short arrow) is infiltrated by lymphocytes and eosinophils within the lumen. The relatively normal-appearing native bile duct (white arrow) and artery are seen at right. Inset: C4d immunohistochemical staining shows strong positivity in the portal vein and adjacent inlet vessels. (Allograft needle biopsy, H&E; inset: C4d-specific immunohistochemistry.)

deposits of immunoglobulin G (IgG) or IgM, complement fractions C1q, C3 and C4 and fibrinogen in arterial walls.[33,40] The graft may remain stable in some patients for the first few days, however, possibly because of Kupffer-cell protection against the effects of circulating antibodies.[41] Graft failure within 2–4 weeks is associated with a progressive and marked rise in serum aminotransferase activity. The liver appears mottled and cyanotic at gross examination. Recipients of ABO-unmatched livers may also develop **graft-versus-host haemolysis**, associated with erythrophagocytosis and **Kupffer-cell siderosis**.[42]

A potential role for antibody-mediated rejection is sometimes considered in the ABO-compatible recipient with early or late allograft dysfunction/failure who has high-titre donor-specific antibodies.[43–45] Recent histopathological data on AMR[46] were incorporated at the most recent (2016) meeting of the Banff Working Group to provide guidelines for histopathological criteria for AMR, which include (1) endothelial cell hypertrophy ('hobnail' endothelial cells) involving portal veins, capillaries and inlet venules (periportal sinusoids), accompanied by (2) marginated and/or intraluminal monocytes, neutrophils or eosinophils adherent to and embedded into the endothelium.[46] C4d immunohistochemical staining of paraffin-embedded biopsies should be scored as minimal (<10% portal tracts), focal (10%–50% portal tracts) or diffuse (>50% portal tracts) positivity of portal vein and portal capillaries, with inlet-venule staining usually a feature seen in the highest score (diffuse) category (**Fig. 16.4, inset**).[47] While AMR is uncommon relative to T-cell-mediated acute cellular rejection (discussed in detail in the next section), when AMR is

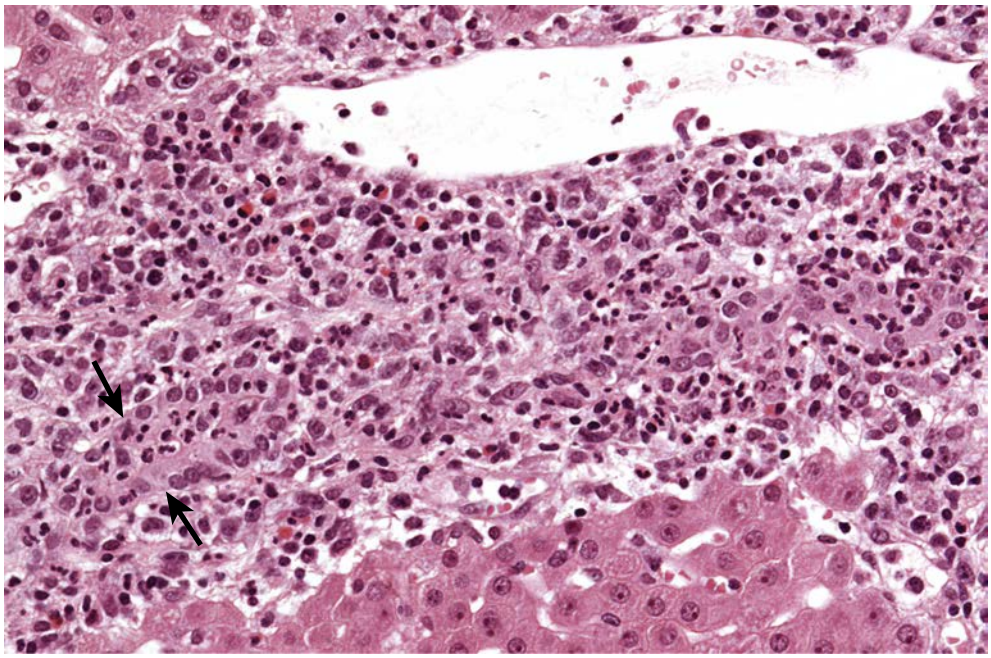

Fig. 16.5 Acute rejection. Heterogeneous portal inflammation consisting of lymphocytes, plasma cells and scattered neutrophils infiltrates the bile duct (between arrows) and the portal-vein branch at top. (Needle biopsy, H&E.)

suspected as a cause of chronic allograft dysfunction and/or fibrosis, the recommended workup should include assessment of donor-specific antibodies, histopathological evaluation and C4d staining.[48]

Acute (cellular) rejection

Acute (cellular) rejection, the most common form of rejection, is a cell-mediated immune injury directed at bile-duct epithelium and the endothelium of portal-vein branches and terminal hepatic venules. This usually occurs within the first month to 6 weeks after transplantation,[49] but may be seen later if immunosuppression is lowered or discontinued. The characteristic histological **triad** of cellular rejection includes **portal inflammation**, **bile-duct damage** and **endotheliitis** (**endothelialitis**). Endotheliitis is not present in all cases. The portal inflammatory lesion is typically heterogeneous, with lymphocytes predominating among plasma cells, neutrophils and, occasionally, large lymphoid cells, some in mitosis (**Fig. 16.5**). Eosinophils are often abundant (**Fig. 16.6**), which is a very helpful diagnostic sign that acute rejection is present.[50,51]

Bile ducts are surrounded and infiltrated by immune cells, and damage to their epithelium takes the form of variation in nuclear size, vacuolation of cytoplasm, regions of cell stratification or cell loss and irregularity of duct outlines (**Figs 16.5–16.7**). Endotheliitis comprises attachment of lymphoid cells to the endothelium of portal-vein branches or terminal hepatic venules, variable degrees of endothelial damage, subendothelial inflammation (**Figs 16.5 and 16.6**) and lifting off of endothelial cells from the underlying vein wall (**Fig. 16.8**). Sinusoidal endotheliitis is occasionally also present, but can be a prominent pattern in certain cases.[52] Mild focal endotheliitis is sometimes found in association with hypoperfusion damage in baseline biopsies, but extensive endotheliitis in the postoperative period is very characteristic of rejection.[34] Necrosis of perivenular hepatocytes, accompanied by endotheliitis of terminal hepatic venules and expansive portal inflammatory lesions involving the periportal parenchyma, is indicative of severe acute rejection.[53] **Central perivenulitis (central venulitis)**—characterised by endotheliitis of

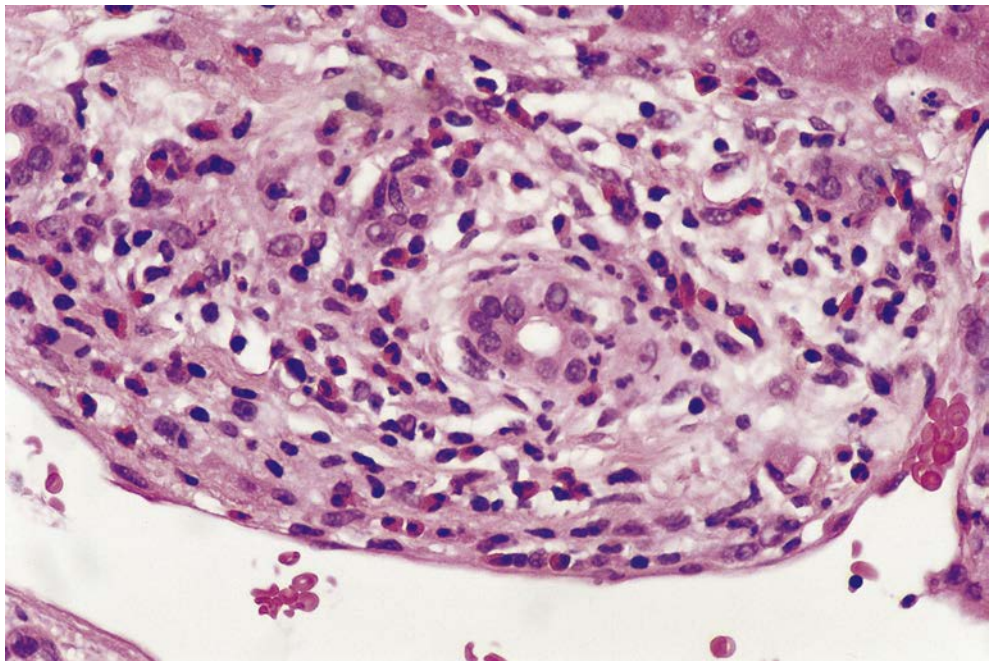

Fig. 16.6 Acute rejection. The portal tract infiltrate is rich in eosinophils. The portal-vein branch at bottom shows endotheliitis, with subendothelial lymphocytes and eosinophils. (Needle biopsy, H&E.)

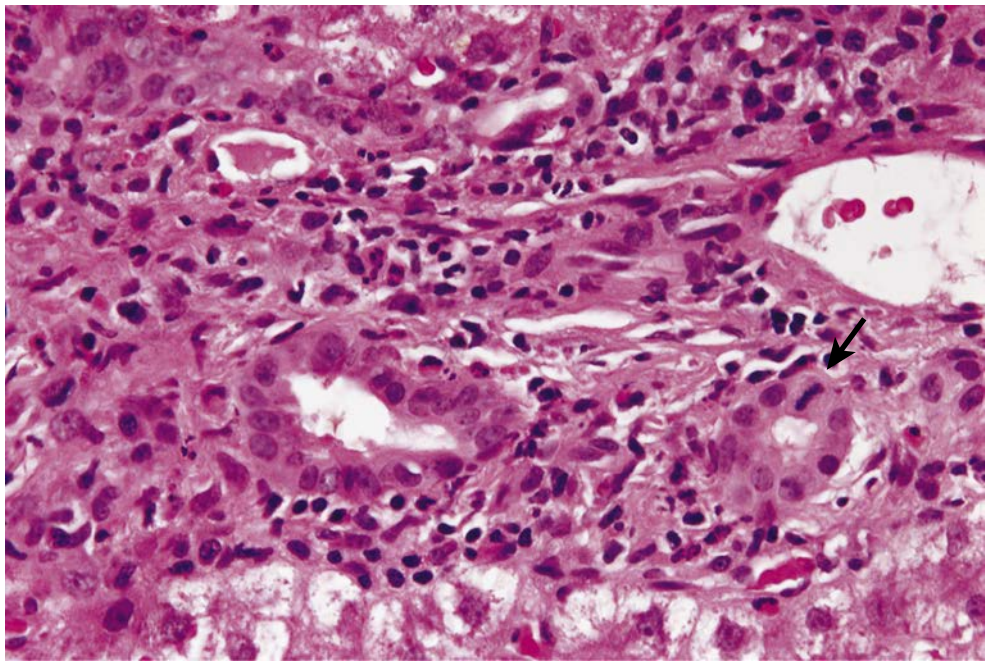

Fig. 16.7 Acute rejection. A damaged bile duct, cut twice in this portal tract, shows irregular epithelium with mild nuclear pleomorphism. Neutrophils are admixed with lymphocytes around and above the duct at left. The duct profile at right shows a mitotic figure (arrow). (Needle biopsy, H&E.) (Case kindly provided by Dr Jurgen Ludwig, Rochester, MN, USA.)

terminal venules, drop-out and apoptosis of nearby hepatocytes (sometimes with focal sinusoidal congestion and dilatation)—often presages later episodes of acute rejection and chronic ductopenic rejection.[54–56] Central perivenulitis is a common expression of rejection in paediatric allografts.[57] It is sometimes the chief manifestation of rejection, with few or no portal tract changes, as **isolated central perivenulitis**[58] in both paediatric and adult

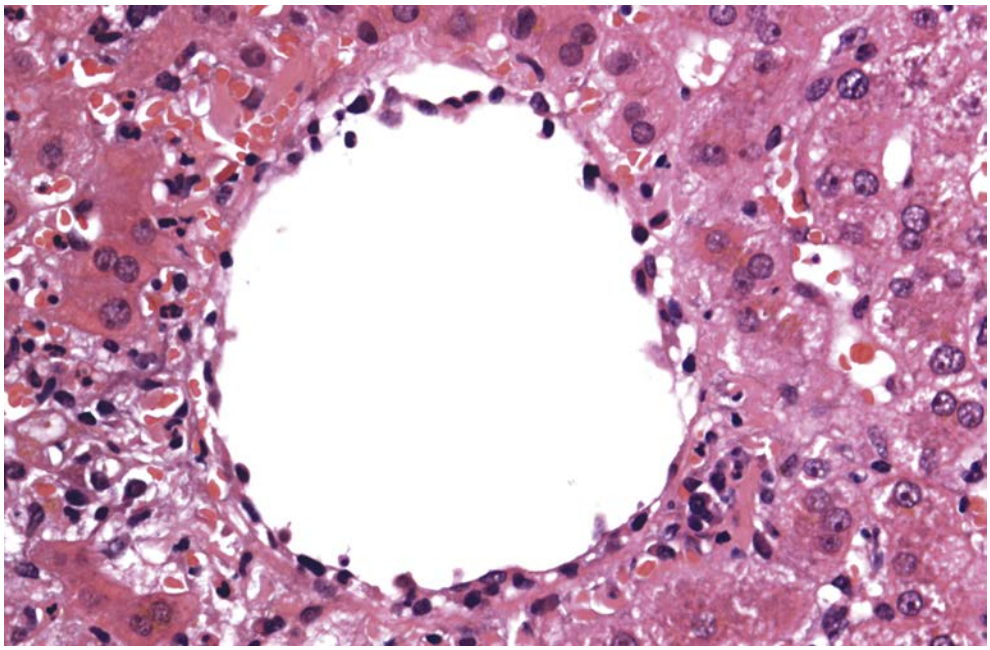

Fig. 16.8 Endotheliitis in acute rejection. An efferent vein shows lymphocytic infiltration of its wall. The endothelium is focally lifted off the underlying vein wall and partially destroyed. (Needle biopsy, H&E.)

allografts, many months or longer after transplantation (see 'Late liver allograft dysfunction' section, later).

Descriptive and semi-quantitative **grading of acute rejection** can effectively be accomplished using the scoring system presented in the Banff international consensus document[53] (**Table 16.1**). Using the semi-quantitative approach of assigning a numerical score to each component of the acute rejection triad, a total **Rejection Activity Index (RAI)** can be conveyed in the biopsy report. Alternatively, a simpler global assessment of the biopsy as showing indeterminate, mild, moderate or severe changes of acute rejection can be used (**Table 16.2**). The choice of grading system, as with grading and staging for chronic hepatitis, should be made after discussion with clinicians.

Chronic (ductopenic) rejection

Chronic (ductopenic) rejection (vanishing bile-duct syndrome) is defined as obliterative vasculopathy and loss of bile ducts occurring 60 days or longer after transplantation.[33,59] The incidence of chronic rejection in liver transplant patients has declined to less than 5% in some series[60–62] as immunosuppression regimens have improved. In most cases, vasculopathy and ductopenia occur together, but in a minority they can be present independently.[63]

The diagnosis of chronic rejection can be problematic even for experienced hepatic pathologists,[64,65] particularly in the early stages.[33] Atrophy, nuclear pleomorphism and pyknosis of small ducts (bile-duct 'dystrophy') often precede frank ductopenia.[66] If such bile duct dystrophic and senescent changes are widespread, clinical jaundice may be prolonged over many weeks and associated with prominent centrilobular cholestasis and hepatocyte ballooning on liver biopsy (**Fig. 16.9**). The centrilobular changes may be mistaken for drug-induced liver injury, but careful inspection of the bile ducts usually clarifies the cause to be pervasive rejection-related duct injury. The presence of ductopenia is established when a formal count of small bile ducts and hepatic artery branches within portal tracts demonstrates loss of bile ducts from over 50% of portal tracts (**Fig. 16.10**). Progressive bile-duct loss results from a destructive cholangitis, which in most cases stems from bouts of acute rejection that are not controlled by immunosuppression. Cytokeratin

Table 16.1 Banff grading scheme for acute rejection.*

Category	Criteria	Score
Portal inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile-duct inflammation damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes, such as increased nucleus-to-cytoplasm ratio of the epithelial cells	1
	Most or all of the ducts are infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolisation of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal luminal disruption	3
Venous endothelial inflammation	Subendothelial lymphocytic infiltration involving some, but not most, of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

Note: Total score = sum of components. Criteria that can be used to score liver allograft biopsies with acute rejection are as defined in the World Gastroenterology Consensus Document.
*The Rejection Activity Index (RAI) is the sum of the scores for each of the three components of acute rejection. RAI ≥ 4 (mild), RAI ≥ 6 (moderate or severe).
Reproduced from International Panel. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997;25:658–663.

immunostaining may help identify remnants of bile-duct epithelium.[37] Portal tract hepatic arterioles may also be lost.[59] Over time, portal inflammation becomes sparse and bile ducts disappear from the majority of portal tracts, usually without a ductular reaction[59] (**Fig. 16.10**). Episodes of acute rejection with increased inflammation and endotheliitis may develop superimposed on changes of chronic rejection. The pathology report in chronic rejection should therefore include consideration of the following points[59]: (1) whether acute rejection is present; (2) the degree of bile-duct loss in portal tracts; (3) the presence of perivenular necrosis or fibrosis; and (4) the degree of hepatic arteriole loss in relation to the total number of portal tracts.

The presence of obliterative vasculopathy (rejection arteriopathy) may be more difficult to demonstrate on needle biopsies, because the characteristic subintimal accumulations of foamy histiocytes and myointimal cells predominantly affect the large-calibre arteries of the liver hilum[63,67] (**Fig. 16.11**). However, foam-cell lesions can sometimes be demonstrated in medium-sized portal arterioles present in biopsies and occasionally in portal veins and sinusoids (**Fig. 16.12**). The presence of arteriopathy in most cases must be inferred when

Table 16.2 Descriptive terminology for acute rejection

Global assessment*	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute rejection (see text)
Mild	Rejection infiltrate in a minority of the triads that is generally mild and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all of the triads
Severe	As above for moderate, with spillover into periportal areas and moderate-to-severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

Note: Global assessment of rejection grade is made on a review of the biopsy and after the diagnosis of rejection has been established.
*Verbal description of mild, moderate or severe acute rejection could also be labelled as grades I, II and III, respectively.
Reproduced from International Panel. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997;25:658–663.

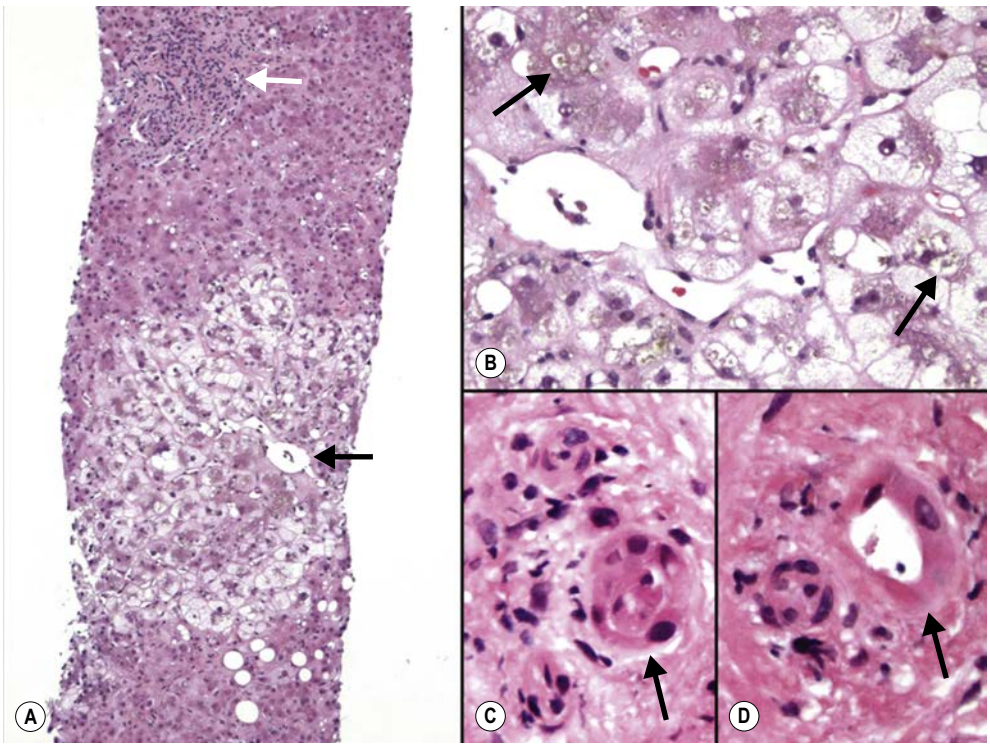

Fig. 16.9 Prolonged acute rejection with extensive bile-duct injury, centrilobular cholestasis and hepatocyte ballooning. A: Marked cholestasis and hepatocyte ballooning are present in the centrilobular region (black arrow) and reflect the extensiveness of rejection-related bile-duct injury. The portal tract at top (white arrow) shows rejection-related inflammation. **B:** Marked cholestasis within bile canaliculi (arrows) and hepatocyte has resulted in prominent liver-cell ballooning. **C:** The interlobular bile duct (arrow) is dysmorphic, with altered nuclear size and chromaticity, as well as dys-polarity. **D:** This severely dystrophic (senescent) bile duct (arrow) shows a highly simplified structure composed of only a few cells with disparate nuclear features. (Allograft needle biopsy, H&E.)

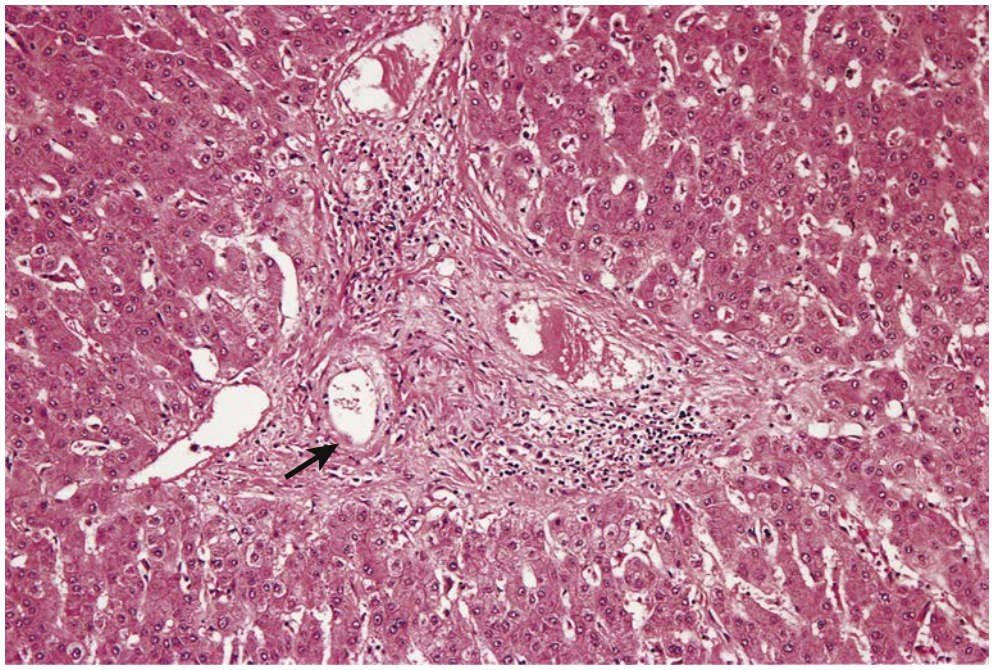

Fig. 16.10 Chronic (ductopenic) rejection. A hepatic artery branch (arrow) is present in the portal tract, but the corresponding interlobular bile duct has disappeared as a result of rejection. A sparse lymphocytic infiltrate remains. (Explanted donor liver, H&E.) (Case kindly provided by Dr Jurgen Ludwig, Rochester, Minnesota, United States.)

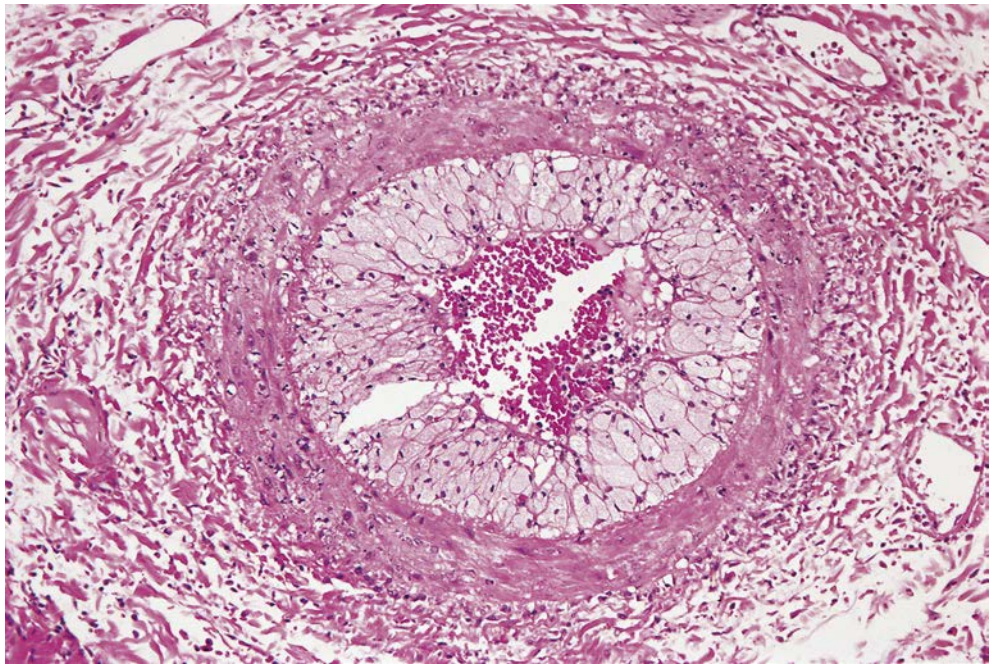

Fig. 16.11 Rejection arteriopathy. A hilar artery from a transplant liver removed because of rejection shows an accumulation of subintimal foam cells. (Explanted donor liver, H&E.) (Case kindly provided by Dr Jurgen Ludwig, Rochester, Minnesota, United States.)

perivenular ischaemic necrosis and fibrosis are seen in liver biopsies obtained in the appropriate time frame of chronic rejection. Demonstration of perivenular necrosis in repeated biopsies indicates a poor prognosis.[68] Mismatch of recipient and donor histocompatibility antigens, activation of the complement membrane attack complex and persistent cytomegalovirus (CMV) infection in the allograft have been invoked in the pathogenesis of bile-duct loss and arteriopathy.[37,69–73]

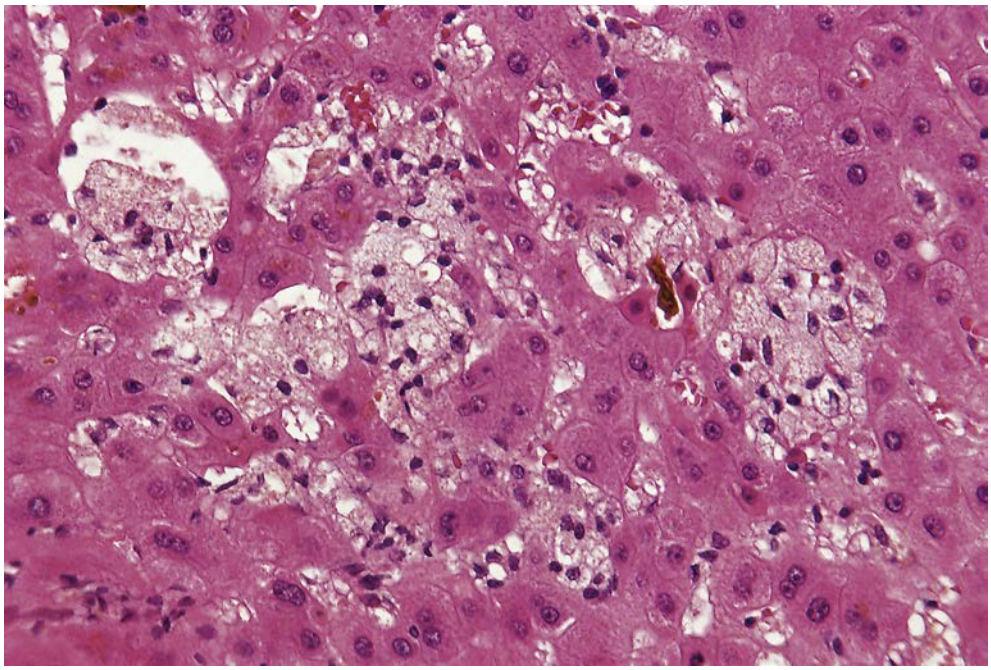

Fig. 16.12 Sinusoidal foam cells in transplant rejection. Months after transplantation, foam cells may be deposited in large-calibre arteries and also within hepatic sinusoids. (Needle biopsy, H&E.)

Chronic rejection usually leads to irreversible graft failure, although some patients may recover.[37,74] The late stage characteristically shows marked cholestasis and bile-duct loss, portal and periportal fibrosis, perivenular fibrosis and variable numbers of bridging fibrous septa linking portal tracts or central veins to portal tracts. Cirrhosis develops after liver transplantation in only a minority of patients and is typically due to recurrent or acquired viral hepatitis, rather than chronic rejection[75] (see 'Recurrent disease' section, later).

Other causes of graft dysfunction

Infection

CMV is a common pathogen in liver allografts; most cases of CMV hepatitis occur 4–8 weeks after transplant.[76] Typical intranuclear and cytoplasmic CMV inclusions (**Ch. 15**) can be found in hepatocytes, bile-duct epithelium (**see Fig. 15.5**) and endothelial cells. CMV infection should be suspected when small **microabscess**-like foci of necrosis with an infiltrate of neutrophils are present (**Fig. 16.13**). Smaller collections of parenchymal neutrophils (**mini-microabscesses**) are occasionally seen in patients without CMV infection, apparently without adverse effects on the graft.[77] CMV infection may also lead to formation of epithelioid granulomas. Immunohistochemical staining for CMV antigens is a sensitive method of demonstrating occult infection.[78]

Epstein–Barr virus infection should be considered if portal tracts and sinusoids contain a preponderance of atypical lymphocytes and immunoblasts.[79–81] The possibility that as yet unidentified hepatitis viruses may cause posttransplantation liver dysfunction has been considered.[82]

Infection by **Gram-negative bacilli** may produce hepatocellular and canalicular cholestasis or, with sepsis, the more unusual picture of inspissated bile in periportal bile ductules (bile ductular cholestasis; **Ch. 15 and Fig. 15.12**). Cholestasis due to infection and/or sepsis must be distinguished from that seen in bile-duct obstruction and rejection.

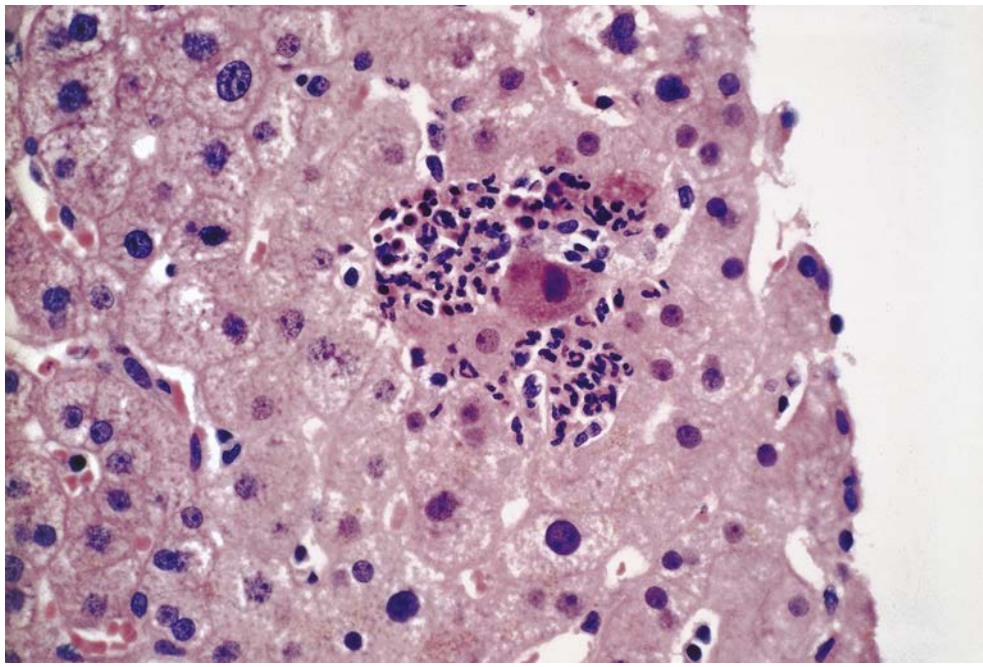

Fig. 16.13 Cytomegalovirus hepatitis. A microabscess-like cluster of neutrophils surrounds a hepatocyte with a smudged intranuclear inclusion. (Needle biopsy, H&E.)

Assessment of portal tract changes as well as results of microbiological studies are important in making these distinctions. Culture and special stains of liver biopsy specimens that show microabscesses or granulomas are the best means of documenting **bacterial**, **fungal or other infections.**[83]

Biliary obstruction

Perihilar bile leaks (bilomas), anastomotic strictures[84] and, less commonly, bile cast syndrome[85] may develop following transplant, with associated cholestasis and portal tract changes of obstruction (**Ch. 5**) on biopsy. There may be residual portal and periportal fibrosis with a ductular reaction after prior episodes of obstruction and decompression by biliary stenting which should be taken into account when interpreting later allograft biopsies.

Thrombosis

Thrombosis of the hepatic artery[86,87] or portal vein[88a] (the latter particularly in children) may develop within the first few weeks or months of transplantation, leading to infarction of the liver (**Ch. 12**). Needle biopsy specimens may not be representative owing to the irregular distribution of infarcted liver parenchyma. Other less specific changes may be seen in needle biopsies and/or explants after arterial thrombosis, such as biliary material within portal vein branches, bile ductular cholestasis and bile-laden Kupffer cells.[88b] Thrombosis, stricture or foam-cell arteriopathy of perihilar arteries may cause necrosis, stricture or cholangiectases of perihilar bile ducts due to impaired duct perfusion.[89] Liver biopsy in such cases may show features of biliary obstruction.[89]

Drug toxicity

The therapeutic regimen for immunosuppression in liver transplant patients includes several potentially hepatotoxic agents. **Azathioprine** hepatotoxicity has been reported primarily in renal transplant patients (see 'Renal transplantation' section, later). Elevated activities

of serum aminotransferases with **sinusoidal congestion** and **perivenular necrosis** have been described in liver transplant patients treated with this drug,[90] and **veno-occlusive disease** elsewhere in the graft should be suspected, even if not demonstrated in the biopsy sample. There may also be **fibrosis of terminal hepatic venules**, particularly in patients with cellular rejection and endotheliitis.[91] **Ciclosporin** may cause **cholestasis**[92] by inhibition of adenosine triphosphate (ATP)-dependent bile-salt transport.[93,94] Although a similar mechanism of cholestasis obtains for **FK 506**, hepatotoxicity is rare, probably due to the lower dose of FK 506 required for immunosuppression.[95,96]

Immunosuppression withdrawal

Titration downward and cessation of immunosuppression have been undertaken in some transplant recipients with apparently stable allografts in the hope of achieving **operational tolerance** (defined as 'a phenotype of tolerance with an immune response or deficit that has no significant clinical impact'[97]). Because rejection and other changes may develop during the withdrawal process,[97–100] liver biopsy in this setting remains diagnostically important. Protocol pre-weaning biopsies are recommended as a critical baseline for determining candidacy for immunosuppression withdrawal and for monitoring its complications and outcomes.[97]

Recurrent disease

Many of the diseases for which liver transplantation is performed have the potential to recur,[101] including viral hepatitis, malignant tumours, alcoholic and non-alcoholic steatohepatitis,[102–104] Budd–Chiari syndrome and variants of veno-occlusive disease,[105] autoimmune hepatitis (AIH), PBC and primary sclerosing cholangitis (PSC).[106–111] The diagnosis of recurrent disease on liver biopsy can be controversial because some of these pretransplant disorders have histopathological features which overlap with those seen in rejection or posttransplantation biliary obstruction.

Recurrent viral hepatitis in patients transplanted for severe liver disease due to hepatitis B, C and D has diminished substantially because of effective anti-viral therapy, with relatively low recurrence rates of approximately 5% for hepatitis B and 10-15% for hepatitis C.[112-115] In those with **recurrent hepatitis B**, there are varied histopathological expressions including acute hepatitis, chronic hepatitis, cirrhosis, and a carrier state with minimal histological disease.[116,117] Recurrent hepatitis B may evolve from chronic hepatitis to cirrhosis within a year after transplantation.[118] Hepatitis B and D (delta) antigens can be demonstrated by immunohistochemistry in allografts as early as 1–3 weeks after transplantation.[118] For patients with **co-infection by hepatitis B and hepatitis D** in the native liver, recurrence may follow a variable course. In some patients, delta virus recurs without demonstrable hepatitis B virus (HBV) replication (absence of HBV core antigen on immunohistochemistry) or histological evidence of hepatitis.[119] Once HBV replication recurs and core antigen is present in the allograft, chronic hepatitis may then be seen on biopsy.[120]

A minority of patients with recurrent HBV infection may show the distinct histological features of **fibrosing cholestatic hepatitis (FCH)**, including large numbers of ground-glass inclusions, 'cytopathic' liver-cell hepatocyte ballooning, cholestasis, periportal ductular reaction and a network of periportal and sinusoidal fibrosis[121–125] (**Figs 16.14 and 16.15**). This pattern of disease recurrence is associated with high serum HBV DNA levels, extensive immunohistochemical expression of HBV surface and core antigens and a high rate of graft failure. FCH is also seen in liver allografts of individuals with recurrent hepatitis C virus infection. Since FCH develops because of immunosuppression, it is not surprising that FCH may occasionally be seen in heart[126], kidney[127] and bone marrow[128] transplant recipients with underlying chronic hepatitis B and/or C. Very rarely, immunocompetent, non-transplanted individuals with chronic hepatitis B who are being treated with antiviral agents show a flare of hepatitis with histological features of FCH after a lapse of therapy.[129]

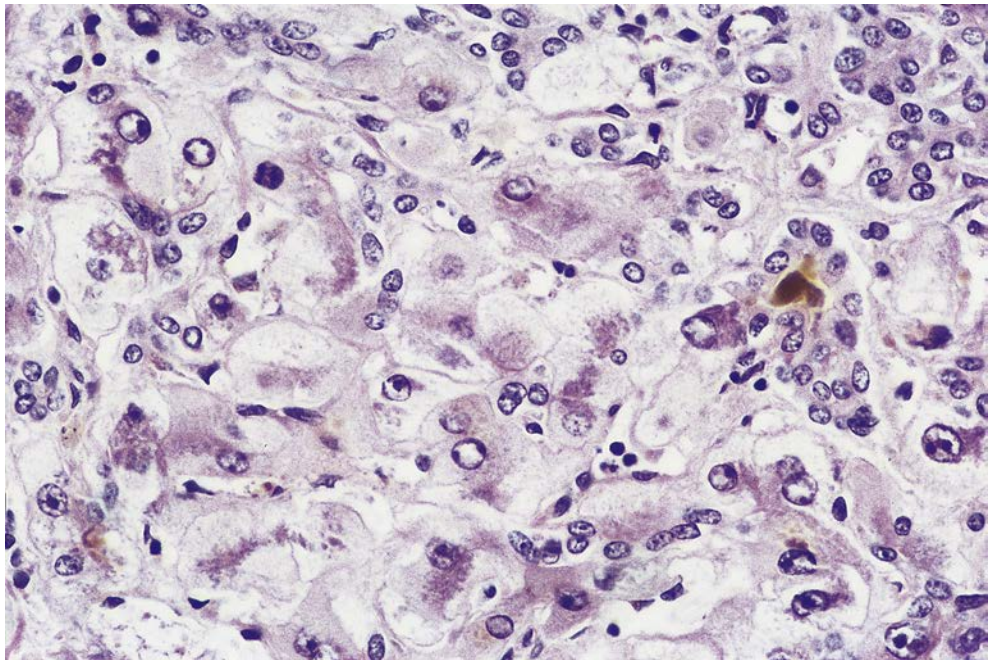

Fig. 16.14 Fibrosing cholestatic hepatitis. Hepatocytes are swollen and many contain ground-glass inclusions. A bile thrombus is seen to the right of centre. (Explanted donor liver, H&E.) (Case kindly provided by Dr Bernard Portmann, London, United Kingdom.)

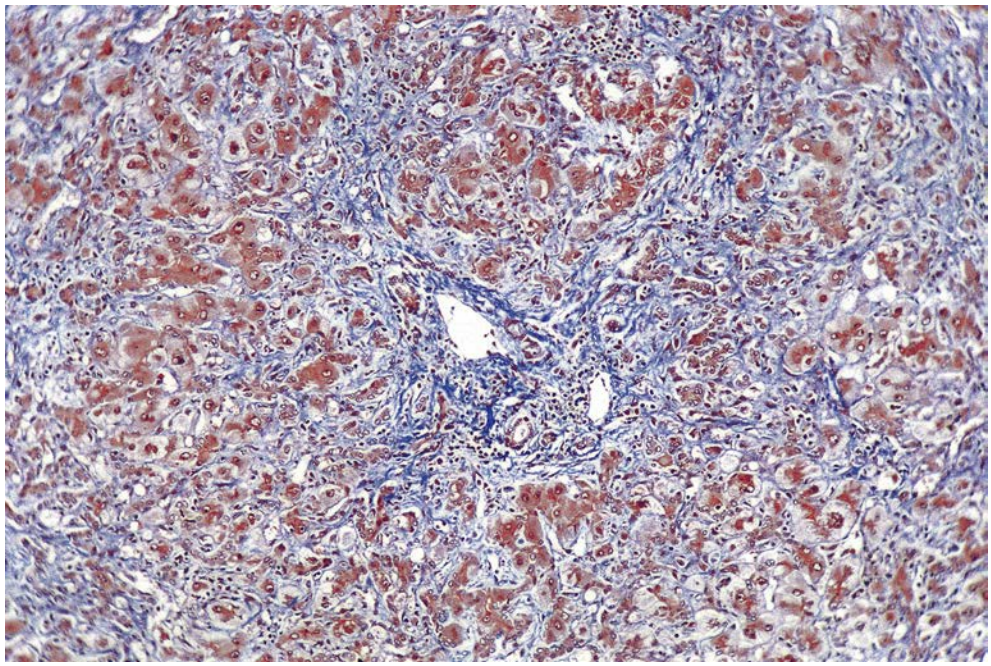

Fig. 16.15 Fibrosing cholestatic hepatitis. Trichrome stain from the case depicted in **Fig. 17.12** shows an intricate network of fibrosis emanating from the portal tract (centre). (Explanted donor liver, trichrome.) (Case kindly provided by Dr Bernard Portmann, London, United Kingdom.)

The risk of **recurrent hepatitis C** has significantly been controlled with the administration of DAAs before or after transplantation. Prior to the availability of DAAs, the histological evolution of HCV infection of the allograft was chronologically relatively well delineated, with this sequence of events: (1) numerous lobular hepatocellular apoptotic

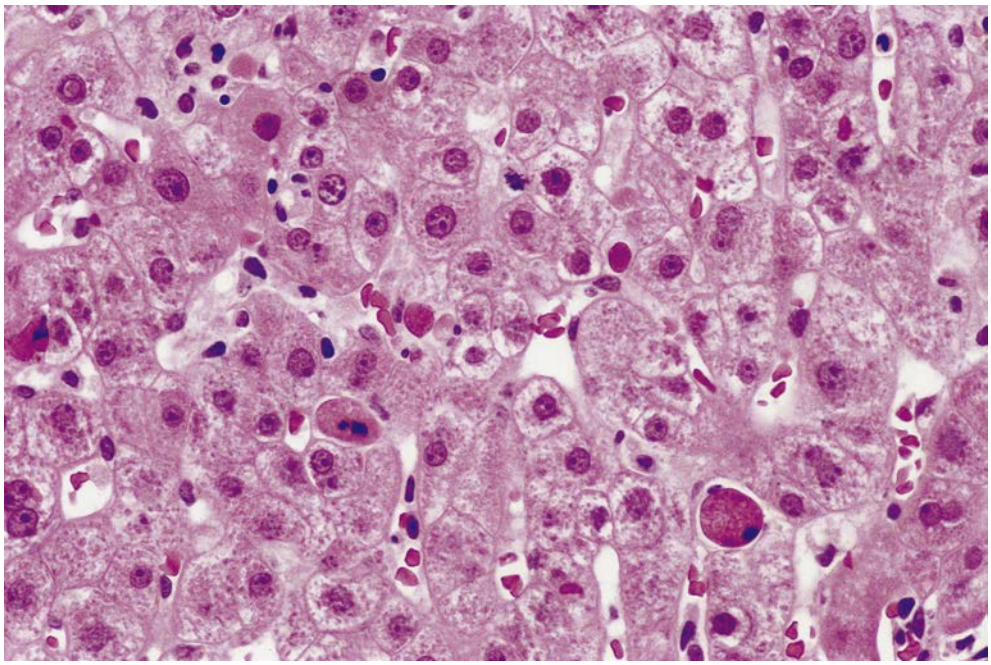

Fig. 16.16 Early recurrent hepatitis C after transplantation. The numerous acidophilic (apoptotic) bodies shown here were the earliest histopathological evidence of recurrent hepatitis C in this case. (Needle biopsy, H&E.)

bodies form, with scant inflammatory cell reaction, within days to a few weeks of transplantation and re-infection (**Fig. 16.16**); (2) a period of lobular hepatitis ensues for several months with an admixture of apoptosis and necroinflammatory foci; and (3) at 6 months and later, the standard features of a chronic hepatitis are recapitulated.[130–134] Steatosis was a feature seen specifically with HCV genotype 3 re-infection.[135] Severe forms of recurrent HCV infection such as fibrosing cholestatic hepatitis C (FCH-C) are now largely an historical footnote because of antiviral therapy for HCV. The histology of FCH-C resembles that seen in HBV infection, including portal and periportal fibrosis and ductular reaction, but features marked hepatocyte ballooning and parenchymal cholestasis in lieu of the HBV-specific ground-glass inclusions.[136–139] Even for the rare case of FCH-C today, DAAs offer improved outcomes.[137,138] Occasional cases of recurrent chronic hepatitis C that were treated with interferon or in association with immunosuppression reduction[133,140–143] showed a plasma cell hepatitis with a preponderance of plasma cells in portal and periportal regions resembling AIH, often with accompanying lymphoplasmacytic central perivenulitis. This pattern was a variant form of *alloimmune* rejection,[144] with a worse outcome.[140] Such complex cases warrant discussion with the clinical team. Distinction of recurrent chronic hepatitis C from acute rejection is often a considerable diagnostic problem,[125,142] especially compounded when evidence of both processes is present in a given biopsy. Many histological parameters must therefore be evaluated (**Table 16.3**). The major pathological process should be emphasised in the pathology report whenever possible.

Following transplantation for **PBC**, serum antimitochondrial antibodies may persist or recur, liver function tests (particularly serum alkaline phosphatase activity) may worsen and liver biopsy may demonstrate recurrent damage to bile ducts.[143] Florid bile-duct lesions and adjacent epithelioid granulomas are the most useful histological signs of recurrent disease. Ductular reaction and progressive copper deposition are other helpful features. There may also be portal lymphoid aggregates and mononuclear inflammation as well as ductopenia, but these can also be seen in HCV infection and rejection. If there is

Table 16.3 Comparative features of acute rejection versus recurrent chronic hepatitis C

Feature	Acute rejection	Recurrent hepatitis C
Lobular necroinflammation	No	Yes
Apoptotic bodies	Few	Many
Cholestasis	Mild	May be marked
Interface hepatitis	No (unless severe)	Often
Lymphoid aggregates/follicles	No	Yes
Portal inflammation	Heterogeneous	Lymphocytes, plasma cells
Fat	No (except with corticosteroid therapy)	Yes (genotype 3 especially)
Ductular reaction	No	Variable (common in cholestatic type)
Central venulitis	Often, diffuse	Uncommon, focal
Bile-duct damage	Yes, diffuse	Focal or none
Portal/periportal fibrosis	No	Often

uncertainty, HCV infection should be serologically excluded. Recurrent PBC can progress to cirrhosis within several years.[144] **AIH** with high levels of serum globulins and typical liver biopsy features has also been reported in patients transplanted for PBC.[145] AIH with high biochemical and histological activity pretransplantation may presage recurrence in the allograft.[146]

Recurrence of **PSC** after transplantation is reported[147] but has been controversial, because the radiological and histopathological features of PSC resemble those seen in biliary complications of the transplant procedure, such as biliary stricture due to hepatic artery thrombosis and bile-duct or choledochojejunostomy-anastomotic obstruction.[101] Biopsy features of cholestasis and portal obstructive changes therefore require cautious interpretation in the context of radiological and other data. Fibro-obliterative lesions (**Ch. 5**) are more specific for recurrence,[147] but are infrequently found in biopsies.[107] Perihilar xanthogranulomatous cholangitis in the explanted PSC liver (**Ch. 5**) has been associated with increased posttransplantation morbidity and mortality.[148]

Studies of patients transplanted for AIH are at variance with regard to the incidence of recurrence.[101] An abrupt rise in serum aminotransferases, detectable autoantibodies, hypergammaglobulinaemia and portal inflammation with interface hepatitis on biopsy are consistent with recurrent disease. Lobular hepatitis may be the first sign of recurrence.[82] In children, recurrent AIH may be an aggressive disease resulting in cirrhosis and retransplantation.[149]

Recurrence of **alcoholic** or **non-alcoholic fatty liver disease** may be manifested by steatosis and steatohepatitis after transplantation.[150] Immunosuppression agents (e.g. corticosteroids, calcineurin inhibitors) and weight gain contribute to recurrent or new metabolic syndrome in adults and in children.[151–155] Steatosis, steatohepatitis and cirrhosis also are seen after transplantation for progressive familial intrahepatic cholestasis type 1.[156]

De novo autoimmune hepatitis

Children and adults who have undergone liver transplantation for conditions other than AIH infrequently develop *de novo* AIH late after transplantation (>6 months to several

years or more after transplantation) accompanied by elevated serum immunoglobulin levels and a variety of autoantibodies.[106–111] The frequency is higher in children (5%–10%) than in adults (1%–2%).[109] The major histological criteria consist of interface hepatitis with substantial activity and abundant plasma cells (**Fig. 16.17**) and plasma cell-enriched centrilobular necroinflammation.[157] Paediatric cases may also show prominent lobular necroinflammation and apoptosis without interface hepatitis or plasma cell–enrichment.[110] Prior to the use of direct-acting antiviral drugs, HCV-positive transplant recipients treated with interferon also developed a 'plasma cell hepatitis' that was considered a form of *alloimmune* rejection, often with a poor prognosis.[158] The Banff Working Group in 2016 has offered the term **'plasma cell–rich rejection'** to describe such cases.[47]

Neoplastic disease

Posttransplant lymphoproliferative disease (PTLD), chiefly B-cell lymphoma in lymph nodes and extranodal sites, is a complication of immunosuppression in patients with organ transplants. Special studies are important in determining whether the PTLD is polymorphic or monomorphic according to the current World Health Organisation classification.[159] B-cell lymphoma in the liver has been reported as early as 2 months after liver transplantation[160] but usually occurs 1 year or more after adult liver transplantation and within a year in children.[159] Lymphoma usually originates in recipient lymphoid

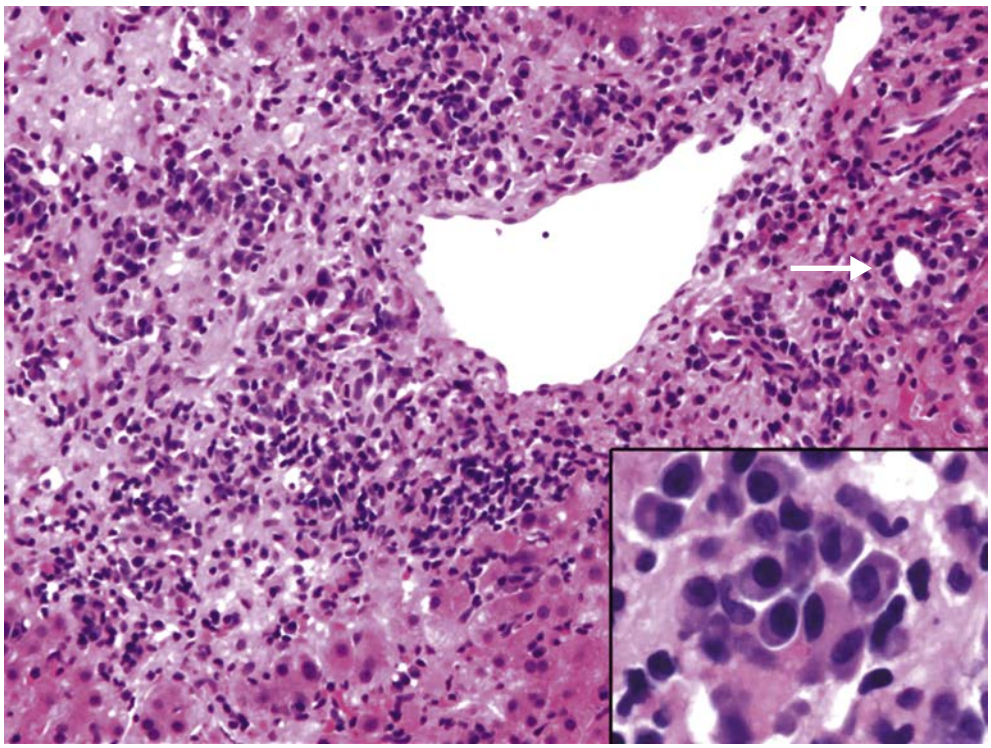

Fig. 16.17 ***De novo*** **autoimmune hepatitis.** Liver dysfunction developed 3 years after liver transplantation for alcoholic cirrhosis in this case, and the allograft biopsy showed marked plasma cell infiltrates (inset) within portal tracts and periportal regions, with extensive interface hepatitis. The bile duct (white arrow) and portal vein branch to its left appear normal. Centrilobular necroinflammation with increased plasma cells was also present. (Allograft needle biopsy, H&E.) (Case kindly provided by Dr Glen Friedman, Las Palmas Medical Center, El Paso, Texas, United States.)

tissue, but rarely it may be derived from donor lymphoid tissue present in the allograft.[161] Hepatic involvement consists of diffuse lymphoma nodules or portal tract infiltration by lymphoma cells (**Fig. 16.18**). Biopsy demonstration of Epstein–Barr virus, which is involved in the pathogenesis of most cases of PTLD,[159] is helpful in distinguishing this from rejection.[162] *De novo*[163,164] or recurrent[165] **hepatocellular carcinoma** has also developed in patients transplanted for chronic hepatitis B and C, even after viral clearance.[166]

Late liver-allograft dysfunction

Most transplant recipients with abnormal liver function tests or symptoms at 1 year or later after transplantation show biopsy changes related to recurrent disease or biliary stricture.[167] However, as with earlier biopsies, several pathological processes may be evident, and helpful histological guidelines for sorting these out have been provided by the Banff Working Group.[47,167] Examination for changes of acute or chronic rejection is always important. Particular attention should be paid to perivenular regions because certain cases of ongoing or late acute rejection may be confined to these areas as **isolated central perivenulitis**[58,168,169] (**Fig. 16.19**). The changes are similar to those of central perivenulitis earlier after transplantation, but there may also be perivenular fibrosis and possible evolution to ductopenic rejection. The degree of terminal venule involvement and associated perivenular hepatocyte drop-out and necrosis in isolated central perivenulitis should be specifically described in the diagnosis.[167]

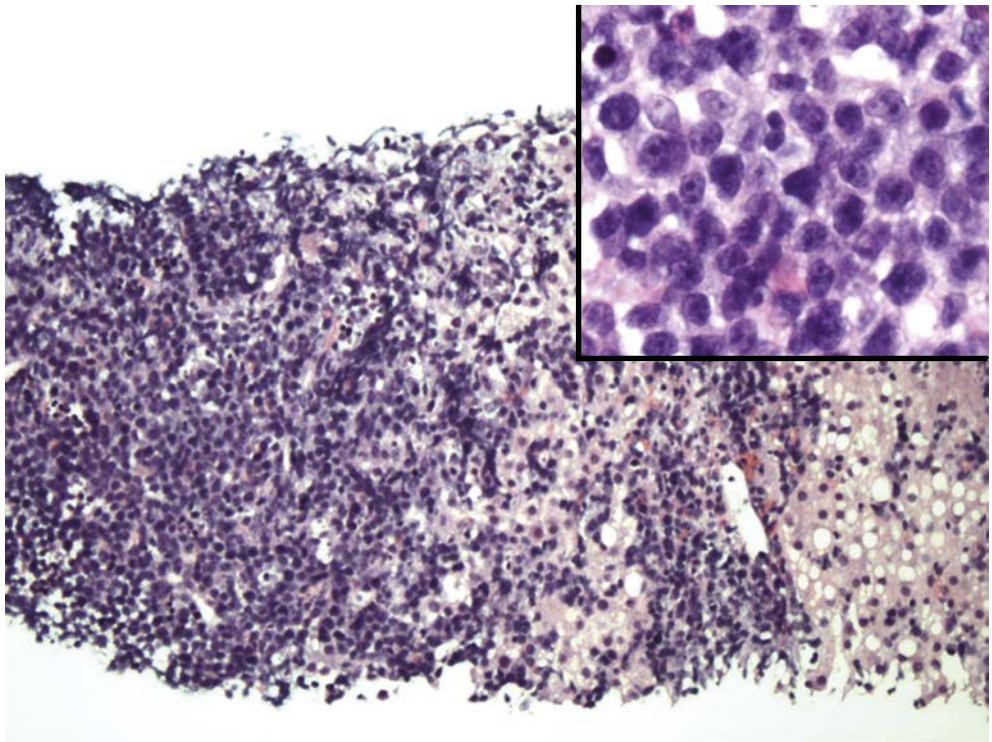

Fig. 16.18 Posttransplant lymphoproliferative disease. The portal tract at left is infiltrated and overrun by a proliferation of lymphoid cells. Inset: The cytological features are consistent with a high-grade, large B-cell lymphoma. Flow cytometry, immunohistochemical staining and gene rearrangement studies are undertaken for further characterisation of the lymphoid infiltrates. (Needle biopsy, H&E.)

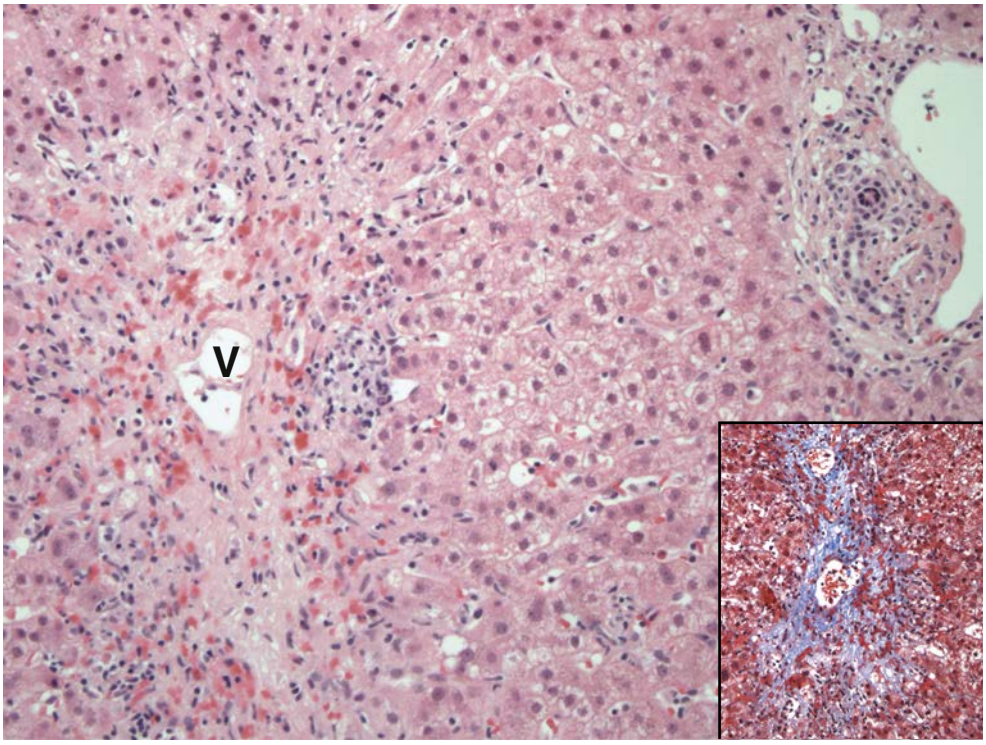

Fig. 16.19 Isolated central perivenulitis. The rejection lesion in this 1-year posttransplant biopsy chiefly involves terminal venules (V) where lymphocytic infiltrates, congestion and perivenular fibrosis are seen. The portal tract at upper right is relatively spared, with only sparse lymphocytes and no bile-duct or portal-vein damage. Inset: Connective tissue stain highlights the collagenous scar surrounding the venule. (Needle biopsy, H&E. Inset: Trichrome stain.)

Late biopsies sometimes show non-specific portal or lobular lymphocytic infiltrates of uncertain aetiology in the absence of more diagnostic changes of rejection. Unexplained periportal fibrosis with histological changes of chronic hepatitis and/or cirrhosis that are unexplained by chronic hepatitis B or C or AIH often prove to be instances of **'idiopathic posttransplantation hepatitis' (IPTH)**, a late, variant form of late cellular rejection seen in adult and paediatric[170] allograft biopsies.[107,171] Portal tract lymphocytic infiltrates with or without interface hepatitis, lymphoid aggregates and variable sinusoidal and/or perivenular endotheliitis are seen (**Fig. 16.20**). Years after transplantation, paediatric liver allograft recipients may demonstrate T-cell-mediated rejection with interface hepatitis and/or portal/periportal fibrosis, despite routinely normal serum liver test results.[172]

Another consideration as a potential cause of late allograft dysfunction with histological features of IPTH is chronic hepatitis E virus (HEV) infection (whether recurrent, reactivated or newly acquired in the donor liver), which should be excluded with serological studies of HEV RNA, as well as IgM and IgG anti-HEV antibodies.[173–176]

Differential diagnosis in transplant biopsies

Most problems in biopsy interpretation after liver transplantation arise in distinguishing rejection from other conditions (**Table 16.4**). It should be kept in mind that rejection and other allograft disorders can coexist. Difficult pathological problems are usually resolved by discussion with clinicians, assessment of viral serologies and microbial culture results

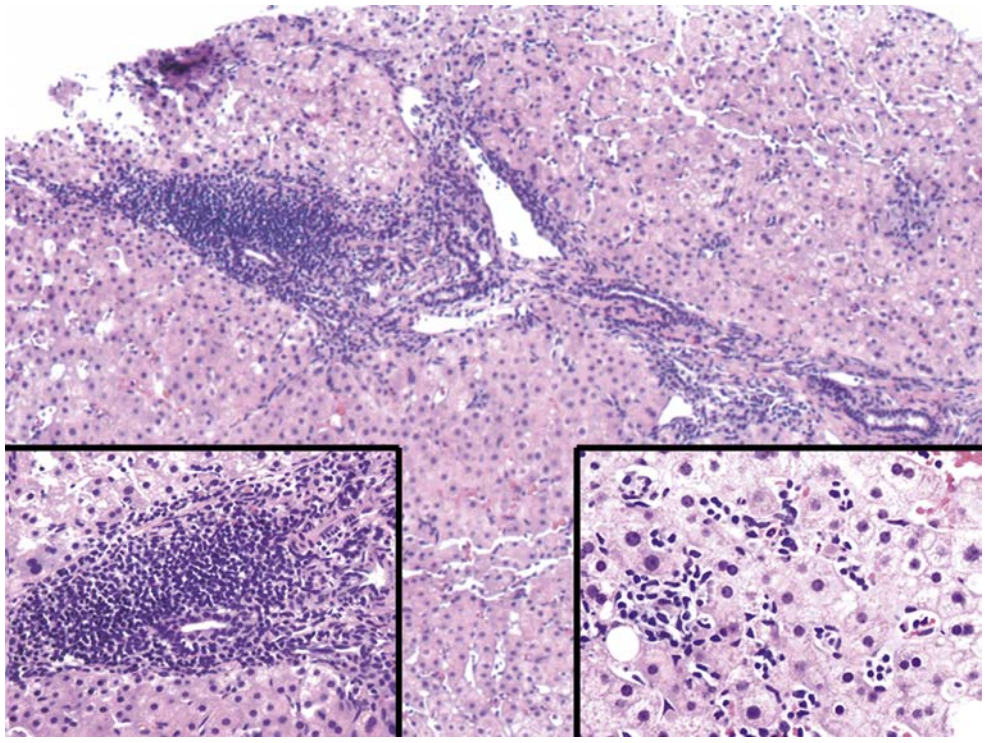

Fig. 16.20 Late liver allograft rejection (idiopathic posttransplantation hepatitis). Biopsies obtained years following liver transplantation may show portal and periportal cellular rejection with features resembling chronic hepatitis, including lymphoid aggregates with mild accompanying chronic inflammation, including interface hepatitis (inset, lower left). Sinusoidal endotheliitis may be prominent (inset, lower right) and sometimes accompanied by hepatocyte apoptosis; these changes can potentially be mistaken for an acute viral or drug-related hepatitis. (Needle biopsy, H&E.)

and review of drug therapy. When necessary, patency of vascular or biliary anastomoses may need to be radiologically demonstrated.

While **endotheliitis** may be seen in several forms of liver disease,[177] when it is found in combination with bile-duct damage and a mixed portal inflammatory infiltrate, the diagnosis of acute rejection is usually clear. Endotheliitis involving central veins (**central venulitis**) sometimes presents diagnostic difficulties.[57] Regular involvement of most central veins in a biopsy specimen favours rejection or the less common development of *de novo* AIH. Central venulitis due to viral and drug hepatitis is more irregular in distribution. **Bile-duct damage** represents a more difficult histological problem because it is a feature seen in rejection, chronic hepatitis C and PBC. Portal tract lymphoid aggregates, numerous apoptotic bodies, interface hepatitis and prominent sinusoidal inflammation support the diagnosis of chronic hepatitis C. As noted earlier, the presence of a granulomatous, destructive cholangitis in a hepatitis C–seronegative patient transplanted for PBC is important evidence of recurrent PBC.

Neutrophils may be seen in the vicinity of damaged bile ducts in rejection (**Fig. 16.5**) and should not be mistaken for evidence of biliary obstruction. Obstruction can usually be excluded if portal tract oedema and ductular reaction are absent. **Eosinophils** are often prominent in rejection but can be seen in fewer numbers in recurrent viral hepatitis or AIH. Subendothelial eosinophils in portal-vein branches favour rejection. Drug hepatitis as a cause of eosinophil infiltrates may need consideration when there is antibiotic prophylaxis with sulpha- agents such as trimethoprim–sulphamethoxazole,

Table 16.4 Differential diagnostic features in transplant biopsies

Histological feature	Rejection	Condition		Biliary obstruction	Ischaemia
		Recurrent HBV	Recurrent HCV		
Cholestasis	+/–	Unusual (except in fibrosing cholestatic hepatitis)	Unusual	Yes	No
Portal inflammation					
Mixed (L, P, N, E)*	Yes	+/–	+/–	No	No
Lymphocytes, plasma cells	+/–	Yes	Yes	No	No
Neutrophils	+/–	No	No	Yes	No
Bile-duct damage	Yes	Unusual	Yes	No	No
Endotheliitis	Yes	Unusual	Unusual	No	Occasional
Zone 3 necrosis	Yes, if chronic	No	No	No	Yes
Sinusoidal inflammation	No	+/–	++	No	No
Apoptotic bodies	+/–	+	+++	No	No

+/– indicates a feature which is not characteristic, but which may sometimes be present.
HBV, HCV, hepatitis B, C virus.
*Mixed portal inflammation includes lymphocytes (L), plasma cells (P), neutrophils (N) and eosinophils (E).

but the parenchymal changes of acute hepatitis usually help to distinguish this from rejection. **Plasma cells** in small numbers are often present in acute rejection infiltrates, but are also seen admixed with lymphocytes as periportal interface hepatitis in recurrent diseases such as chronic hepatitis B or C or AIH. Interface hepatitis with large numbers of plasma cells in clusters in biopsies from transplant recipients without such antecedent native liver diseases suggests possible *de novo* AIH, which should be further substantiated by the presence of serum autoantibodies and elevated γ-globulin level. Posttransplant recurrent chronic hepatitis C with plasma-cell infiltrates (plasma cell hepatitis) was discussed earlier.

Cholestasis may pose significant diagnostic problems because of several potential causes,[28] including biliary obstruction, rejection and sepsis. In biopsies obtained early (1–2 weeks) after transplantation, cholestasis is usually functional in nature. Small-for-size living donor grafts may also develop cholestasis. Cholestasis accompanied by portal oedema and a ductular reaction should prompt an assessment of the biliary anastomosis. Fibrosing cholestatic hepatitis may mimic such biliary obstructive features, but oedema is typically absent and the hepatocyte swelling and apoptosis accompanying the cholestasis usually clarify that there is recurrent severe HBV or HCV infection. In such cases, clinical exclusion of biliary obstruction is also typically undertaken. The distinctive pattern of 'bile ductular cholestasis' is usually associated with sepsis. Rarely, one or two visibly damaged bile ducts may contain inspissated bile in cases of prolonged, refractory rejection.

Steatosis of large-droplet type in posttransplant biopsies may be due to several factors, including corticosteroid immunosuppression and recurrent HCV infection. Genotype 3 re-infection particularly may result in severe fatty change.[124] Considerable fat also develops in allografts of children transplanted for progressive familial intrahepatic cholestasis type 1.[178]

HBV-negative recipients of liver and other transplants occasionally develop **ground-glass-like hepatocellular inclusions** that are periodic acid–Schiff-positive and represent an abnormal form of glycogen.[179–181] These should be distinguished from other types of ground-glass inclusions (Ch. 4).[182]

Renal transplantation

Patients who have undergone renal transplantation are exposed to many viruses capable of causing acute or chronic hepatitis, particularly hepatitis B[183] and hepatitis C.[184] Steatosis, chronic hepatitis and cirrhosis are often found on biopsy.[185] Fibrosing cholestatic hepatitis is infrequently seen in recipients with chronic hepatitis C.[186] There may be lesions related to administration of potentially hepatotoxic drugs such as azathioprine, which may cause cholestasis, veno-occlusive disease, nodular regenerative hyperplasia and other lesions.[90,187–189] Cirrhosis develops in a small proportion of patients. Incrimination of a single aetiological agent is often difficult. Transfusions may cause substantial siderosis involving both hepatocytes and macrophages.[190,191] Vascular lesions following renal transplantation include narrowing or occlusion of efferent veins,[192] peliosis hepatis[193] and non-cirrhotic portal hypertension.[194] Portal hypertension associated with dilatation of sinusoids in acinar zones 2 and 3, with eventual development of fibrosis or cirrhosis, has also been reported.[195]

Patients who have been treated by **haemodialysis** may have birefringent material, probably derived from silicone tubing, in portal tracts. In some instances this material gives rise to a giant-cell or granulomatous reaction.[196–198] Haemodialysis patients often have Kupffer-cell siderosis.

Bone marrow transplantation

Graft recipients may incur liver damage from graft-versus-host disease (GVHD), chemotherapy-related sinusoidal obstruction syndrome/veno-occlusive disease, infection and idiosyncratic drug jaundice.[199] Siderosis is often present because of prior transfusions. **GVHD** involving the liver has varying histological features depending on the stage of evolution.[200–203] The most characteristic features of acute (less than 90 days after transplant) GVHD are **bile-duct damage** and **cholestasis.**[200,201] The ducts often appear attenuated, tapering and stretched lengthwise. The duct epithelium is irregular, with vacuolated or acidophilic cytoplasm, nuclear pleomorphism and multilayering, and increased nucleus-to-cytoplasm ratio[202,204] (**Fig. 16.21**). Lymphocytes infiltrate the portal tracts and duct epithelium, but are sparse. In early GVHD (less than 35 days), duct changes are less apparent and numerous parenchymal acidophilic bodies may be present.[200] Endotheliitis is uncommon, in comparison with acute liver allograft rejection. In **chronic GVHD** (after 90 days) there is progressive bile-duct dystrophy and senescence, ductopenia and portal fibrosis.[205] The clinical and radiological picture may mimic intrahepatic PSC.[206] Rarely, there are parenchymal changes of an acute hepatitis.[207,208] Cirrhosis of biliary type may finally develop.[209] Chemotherapy for leukaemia/lymphoma may damage sinusoidal endothelium, leading to **sinusoidal obstruction syndrome/veno-occlusive disease**[210] (also discussed in Ch. 12). Such cases show variable perivenular or more diffuse sinusoidal congestion with disruption and effacement of endothelium, intravasation of erythrocytes into the space of Disse and patchy fibrosis of sinusoids and terminal venules[211] (**Fig. 16.22**). Eosinophilic cytoplasmic inclusions in hepatocytes of patients dying after bone marrow transplantation have been described.[212]

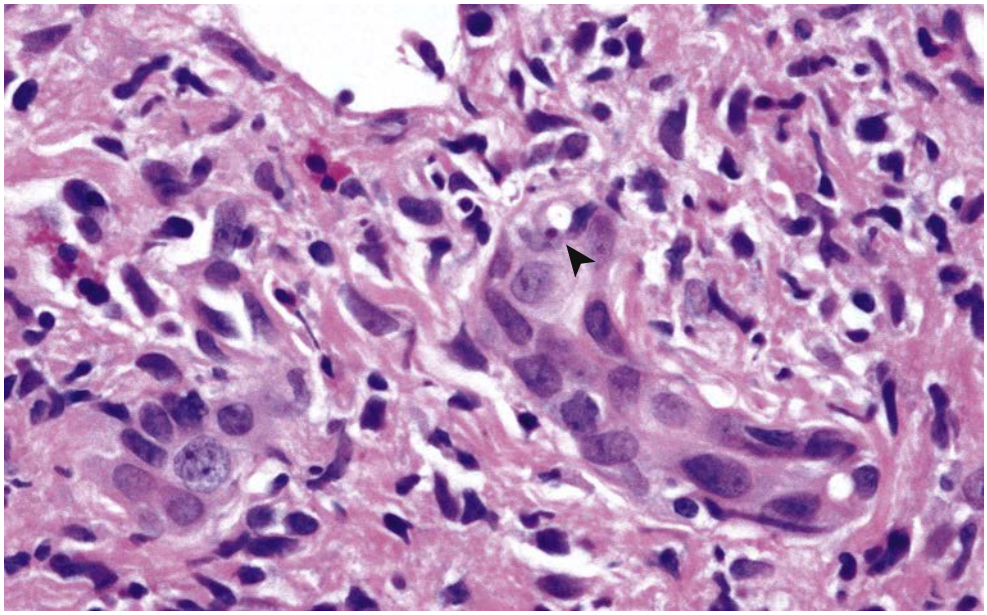

Fig. 16.21 Graft-versus-host disease. The bile-duct epithelium shows dyspolarity and attenuation. A small apoptotic nuclear fragment is seen at top (arrowhead) near an intracellular degenerative vacuole. The surrounding lymphocytic infiltrate is relatively mild. (Needle biopsy, H&E.)

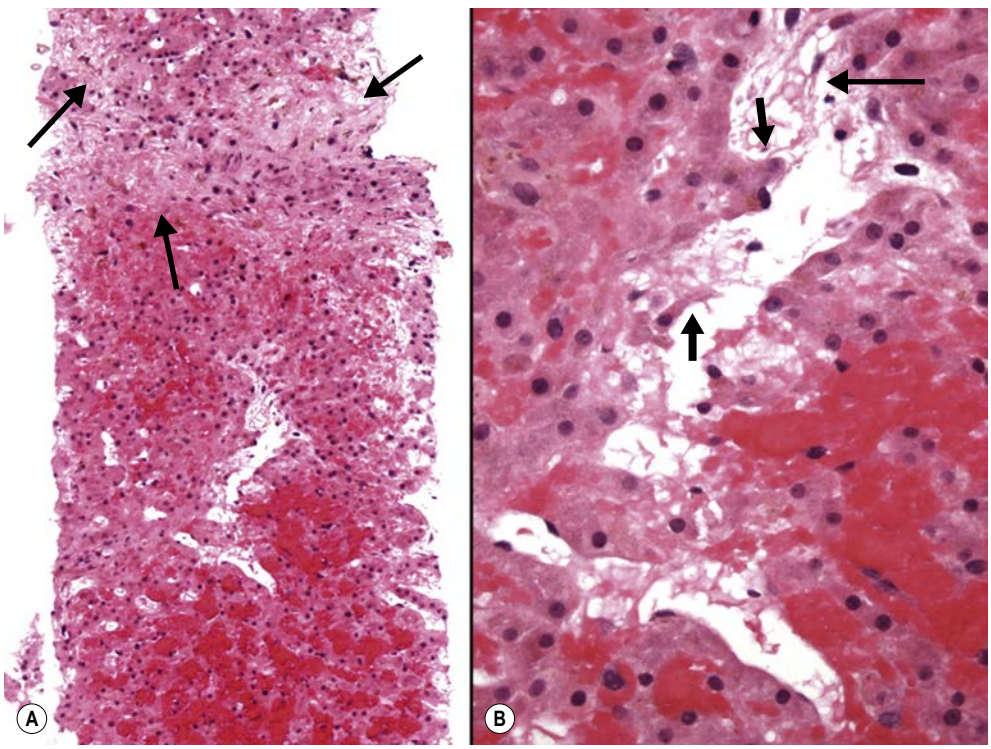

Fig. 16.22 Sinusoidal obstruction syndrome. A: There is marked congestion and several venules, and nearby sinusoids contain plugs of young fibrous tissue (arrows). The terminal venule at centre is injured. The injury was attributed to recent busulphan conditioning chemotherapy prior to hematopoietic stem-cell transplantation for leukaemia. **B:** The endothelium of this terminal venule is partially denuded and detached (long arrow) with extrusion of hepatocytes into the vessel lumen (short arrows). (Needle biopsy, H&E.)

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CHAPTER

17 Electron Microscopy and Other Techniques

Introduction

This chapter will focus primarily on the role of transmission electron microscopy (TEM) in the assessment of liver ultrastructure and disease. It also describes, in brief, the principles and uses of other methodologies. The special conditions required for tissue processing in each of these techniques (**Table 17.1**) should be carefully planned for in advance of obtaining specimens. Use of newer 'molecular fixatives' (in lieu of traditional formalin) for liver specimens is a recent option for improving RNA and DNA preservation while also allowing tissue embedding in paraffin (thereby obviating the need for snap freezing, use of optimal cutting temperature (OCT) compound and other special procedures).[1] Molecular fixatives also produce quality routine and immunohistochemical staining results that are comparable to formalin-fixed specimens.[1] While some of these methods are not universally available in pathology departments, other departments at one's institution or at other centres of investigation may be consulted in cases of particular diagnostic or research interest. Procedures for fixation and processing for TEM are available in several of the papers in the General Reading section at the end of the chapter.

Electron microscopy of liver biopsies

TEM continues to provide important information about the normal cellular and extracellular constituents of the liver and their alterations in disease. Recent interest in the relationships between the various sinusoidal cells of the liver has benefitted from TEM studies,[2,3] as has investigations of hepatic progenitor cells.[4] Data from standard TEM studies can be enhanced by the application of immunohistochemical stains (see the 'Immunoelectron microscopy' section, later), digitised three-dimensional computer reconstructions[5–7] and morphometry. TEM is sometimes limited by the lack of specificity of certain ultrastructural changes and the problem of sampling error in lesions that may not be uniformly distributed. The first of these limitations is well illustrated in cholestasis; various features of cholestasis such as loss of canalicular microvilli are easily recognised under the electron microscope, but many causes produce these changes. Sampling error can sometimes be reduced by the combination of light and electron microscopy in a single instrument.[8]

In diagnostic work, TEM should be seriously considered under five circumstances:

1. *To establish the nature of an inborn error of metabolism.* In a number of storage diseases the ultrastructural changes are diagnostic or give an indication of the type of disease to be considered.[9,10] Specific features are seen, for example, in type II glycogenosis, in Gaucher's disease[11–13] (**Fig. 17.1**) and in Niemann–Pick disease (**Fig. 17.2**). Storage diseases can and should often be diagnosed by other, usually

Table 17.1 Liver tissue processing for various techniques

Technique	Tissue preparation
Transmission electron microscopy	Glutaraldehyde fixation
Scanning electron microscopy	Perfusion fixation; critical point drying; coating with gold or platinum
Immunoelectron microscopy	Glutaraldehyde/paraformaldehyde fixation
Immunoperoxidase of tissue sections	Fixation in 10% neutral formalin or alternative fixative
Immunoperoxidase and immunofluorescence of frozen sections	Snap freeze after embedding in OCT compound*
In situ hybridisation	Snap freeze after embedding in OCT compound*
Flow cytometry	Fresh tissue
Confocal laser scanning microscopy	Snap freeze after embedding in OCT compound*
Laser capture microdissection	Conventional tissue sections for light microscopy
Gene array analysis	Snap freeze in liquid nitrogen*; store at –80° C

OCT, optimal cutting temperature.

*Use of molecular fixatives allows paraffin embedding of tissue in lieu of snap freezing.[1]

biochemical, methods, but even then electron microscopy can reduce the period of investigation by drawing attention to a likely diagnosis. Electron microscopy may show whether a liver-cell pigment is lipofuscin or the pigment of the Dubin–Johnson syndrome (**Fig. 17.3**), and can therefore be helpful when this syndrome is suspected but not fully proved by light microscopy.[14] In some patients with Wilson's disease, characteristic changes may be seen in liver-cell mitochondria (discussed later).

2. *To establish the presence of viral infection.* Electron microscopy of liver biopsies may prove to be important when serological test results or cultures for suspected viral infection are unavailable or incomplete. Both intranuclear and intracytoplasmic virions may be identified by the appearances of their spherical or hexagonal capsids, dense core material, surface envelopes, and paracrystalline and lattice-like arrays. These features can be compared with published micrographs for identification of the candidate virion.[15,16] For example, some adult patients with the unusual finding of giant-cell hepatitis on routine light microscopy have been shown to have paramyxovirus-like particles in the liver as a result of electron microscopic studies of biopsy material.[17,18] Structural changes to the cell may also develop, as in flavivirus hepatic infection by dengue virus, where invagination of portions of the membranes of endoplasmic reticulum (ER) into their own cisternae create distinctive convoluted packets of membrane material within the ER known as 'vesicle packets'.[19] Electron microscopy can also be applied to cell cultures, as shown in a study demonstrating 50–90-nm hepatitis C virions.[20] Glutaraldehyde fixation of biopsy specimens is preferred, but viral particles can also be identified in formalin-fixed tissues which are washed and then processed for electron microscopy.
3. *To establish the nature of a tumour of doubtful histogenesis.* The ultrastructural features of many tumours, including neuroendocrine tumours and malignant melanomas, help in making a firm diagnosis.[21] The more obvious features such as

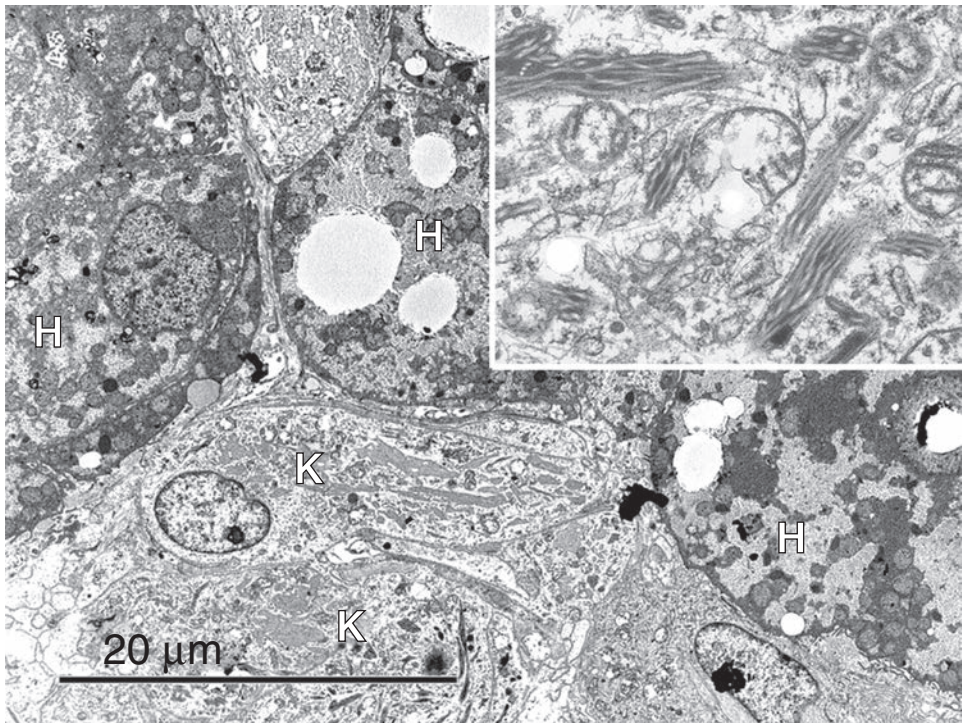

Fig. 17.1 Liver biopsy from a patient with Gaucher's disease and hepatosplenomegaly. Hepatocytes (H) surround a sinusoid with two Kupffer cells (K) that show abnormal tubular structures within the cytoplasm. Inset: The characteristic 'braided tubules' of glycosyl ceramide are present. (Needle biopsy, osmium tetroxide.)

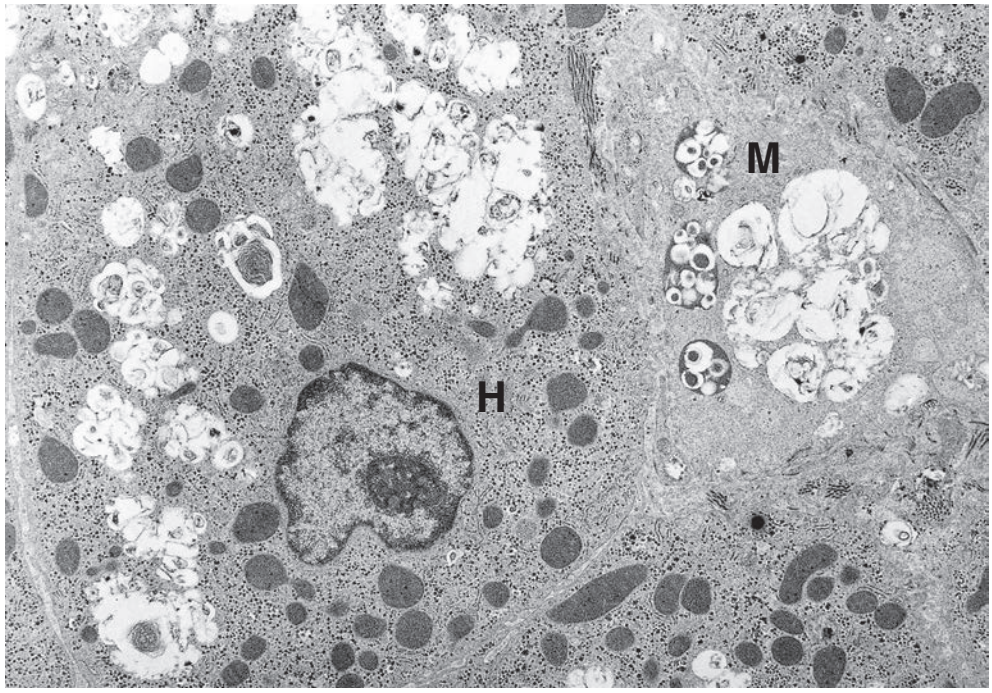

Fig. 17.2 Liver tissue from a patient with Niemann–Pick disease. Macrophages (M) and hepatocytes (H) contain abundant vacuoles in which there are lamellar lipid inclusions. (Needle biopsy, lead citrate; 4600×.)

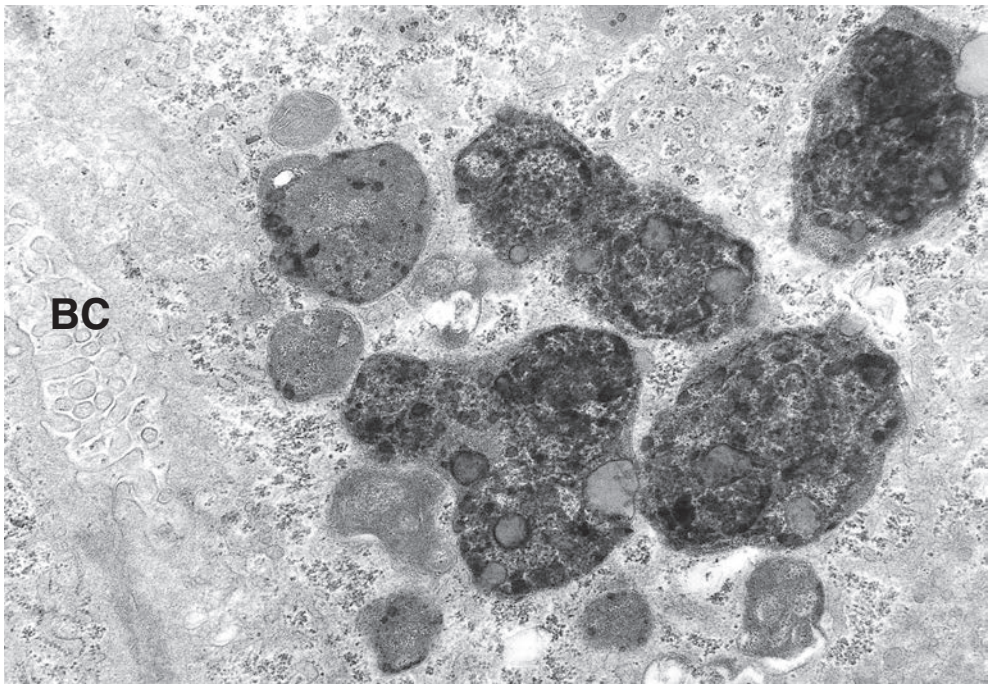

Fig. 17.3 Dubin–Johnson syndrome. Large, characteristically complex dense bodies are seen near a bile canaliculus (BC). (Needle biopsy, lead citrate; 18,900×.)

neurosecretory granules in neuroendocrine tumours survive paraffin embedding; re-embedding of paraffin material for electron microscopy should therefore be considered.

4. *To establish the presence of specific drug-related changes.* In liver damage due to a small number of drugs, including perhexiline maleate[22] and amiodarone,[23–26] hepatocytes contain lysosomes filled with lamellar phospholipid material (**Fig. 17.4**).
5. *To provide material for research.* Electron microscopy offers wide potential for research into human liver disease, and it may be that future research will increase the diagnostic value of electron microscopy in this field. If liver biopsy is performed in a patient who has a disease with potentially helpful or interesting ultrastructural features, small pieces of the specimen can be embedded for electron microscopy and stored indefinitely in block form. The extent to which this is done clearly depends on the resources of the particular laboratory.

Whenever electron microscopy of a liver biopsy specimen is considered, the laboratory should be contacted beforehand and arrangements made for collection and fixation of the specimen at the bedside. Proper processing of the tissue, including optimal fixation, provides the basis for accurate analysis of ultrastructural changes.

The normal liver and examples of ultrastructural changes in disease

The following description of the liver under the TEM is a general one. It should be noted that the quality of fixation will influence the appearance of cells and organelles. The labels in the description of normal liver refer to **Figs 17.5 and 17.6**.

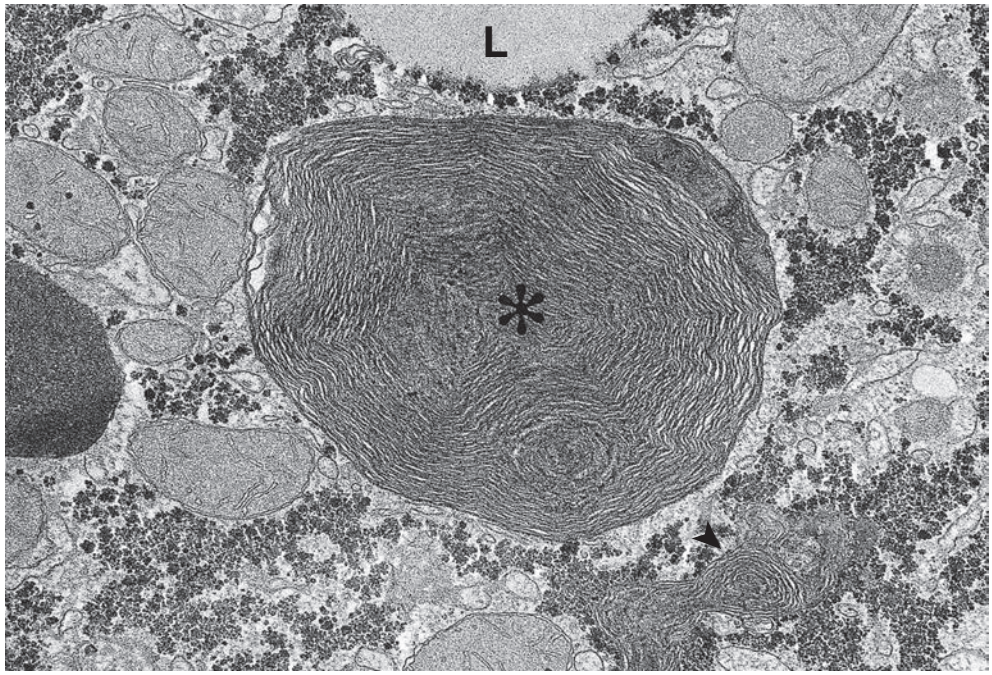

Fig. 17.4 Amiodarone-induced phospholipidosis. An enlarged lysosome (*), resembling a myelin figure, contains densely packed, concentrically arranged osmiophilic lipids, thought to represent drug–lipid complexes. A smaller membranous whorl (arrowhead) is seen in the cytoplasm. L, lipid. (Ferrocyanide, 38,000×.) (Illustration kindly provided by Dr S Poucell and Professor MJ Phillips, Toronto, Canada.)

Several cell types are found in the hepatic lobules. The hepatocytes or parenchymal cells are separated from the sinusoidal endothelial cells by the space of Disse, in which there are collagen fibres and stellate cells, formerly known as perisinusoidal cells, Ito cells or fat-storing cells. Within the sinusoidal lumen are Kupffer cells, the hepatic macrophages and large granular lymphocytes (also called pit cells) with natural killer activity.

Hepatocyte (liver cell, parenchymal cell)

Hepatocytes have similar features in different lobular regions but vary in detailed structure. For example, there are more lysosomes and mitochondria in periportal than in perivenular hepatocytes, while the converse is true for the smooth-surfaced ER. The hepatocyte is a highly polarised cell with surfaces facing the space of Disse, other hepatocytes and the bile canaliculus. The plasma membrane is specialised in these three areas. Many microvilli project into the space of Disse and into the bile canaliculus. This is a potential space formed by two or three hepatocytes in normal liver, and sometimes more in disease. The intercellular membrane of the hepatocyte is relatively smooth and forms several types of intercellular junctions.

The nucleus

The nucleus is normally limited by a double membrane, the nuclear envelope, which is continuous with the rough-surfaced ER. The nuclear envelope has small pores which are thought to serve as a route of communication between the nucleoplasm and the cytoplasm. Within the nucleus there is irregularly distributed chromatin, and a nucleolus is often visible.

Structural changes

Large amounts of monoparticulate glycogen are seen in some adult hepatocyte nuclei in diabetes mellitus and in insulin resistance, in children and also in type I glycogen storage

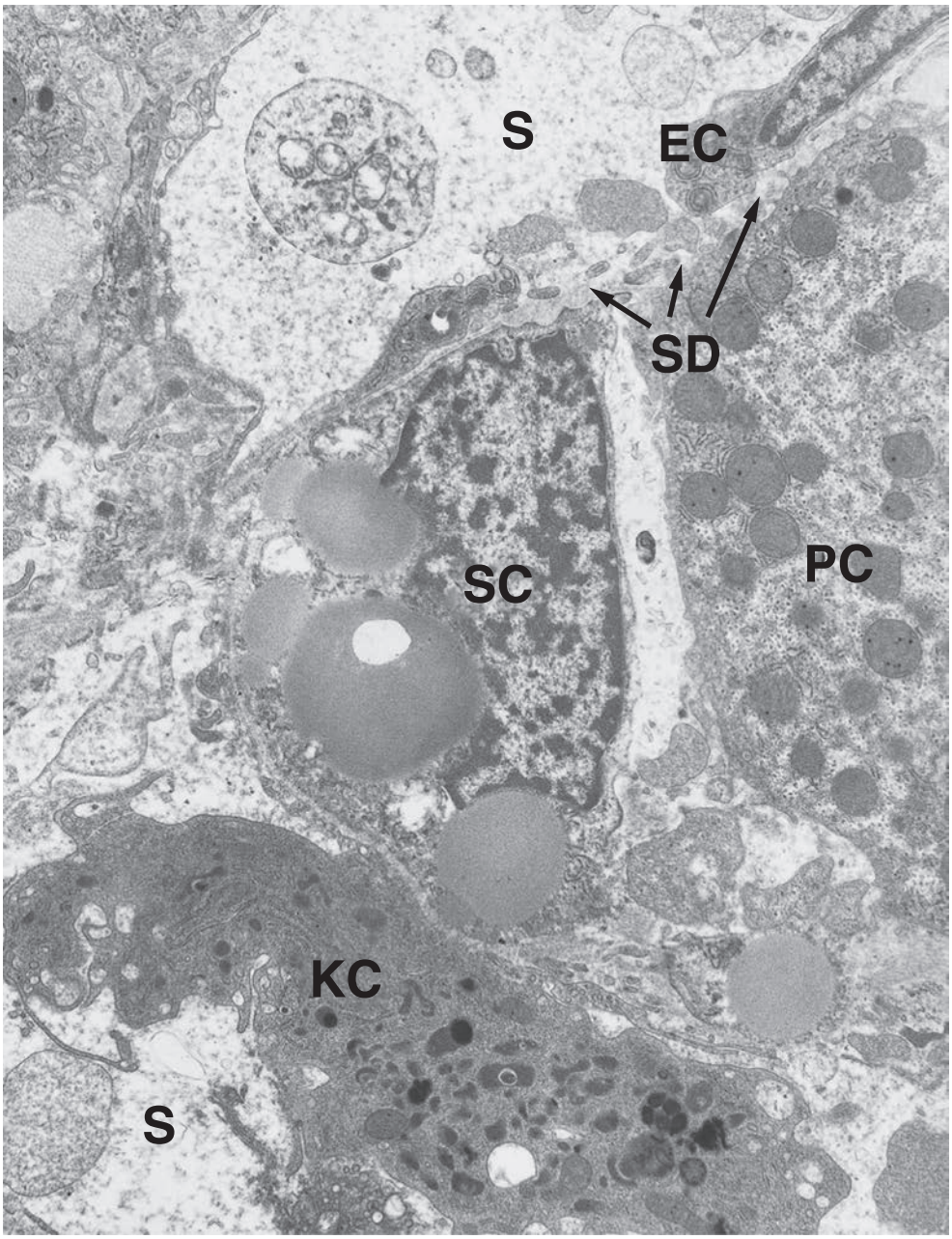

Fig. 17.5 Normal human liver. At the edge of a liver-cell plate the parenchymal cell (PC) is separated from the sinusoidal lumen (S) by an endothelial cell (EC) and Kupffer cell (KC). SC, stellate cell; SD, space of Disse. (Needle biopsy, lead citrate, 10,000×.)

disease. In type B hepatitis, core virus particles are seen (**Fig. 17.7**). Intranuclear virions are also seen in infections due to cytomegalovirus, herpesvirus, echovirus and adenovirus.

Mitochondria

Mitochondria are the sites of oxidative enzyme activity, and are involved in the metabolism of amino acids, lipids and carbohydrates. There are an average of 2200 mitochondria within the hepatocyte.[27] A smooth outer limiting membrane and an inner membrane with

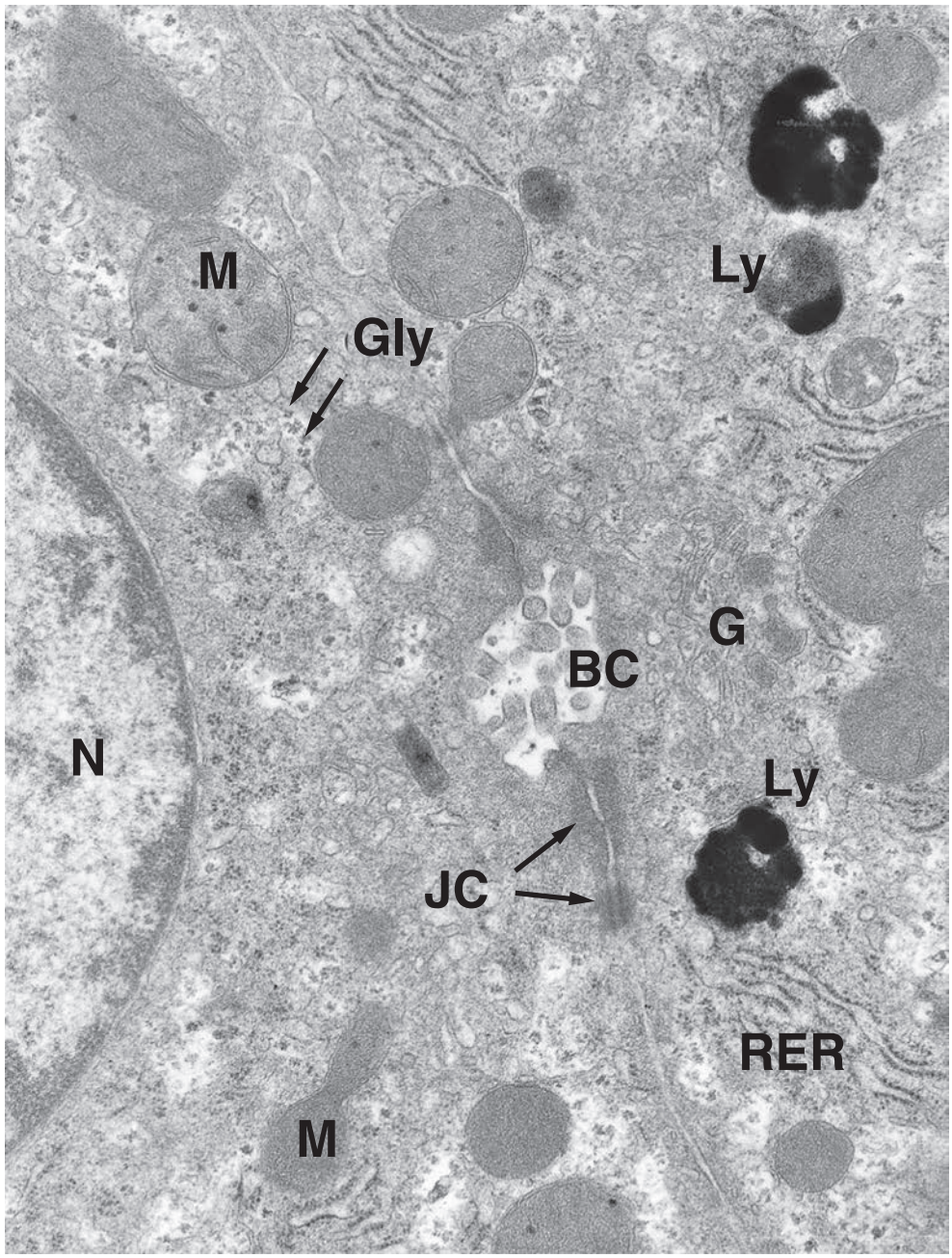

Fig. 17.6 Normal human liver. Two parenchymal cells have formed a bile canaliculus (BC) delimited by junctional complexes (JC) (*arrows*). Lysosomes (Ly) have varying density: the darker ones correspond to lipofuscin, as seen under the light microscope. G, Golgi apparatus; Gly, glycogen; M, mitochondria; N, nucleus; RER, rough-surfaced endoplasmic reticulum. (Needle biopsy, lead citrate, 24,000×.)

deep infoldings, the cristae, give the mitochondria a characteristic appearance. The inner membrane surrounds the mitochondrial matrix which contains many dense granules.

Structural changes

Cristae of atypical shape, crystalline inclusions and enlarged or unusually scanty granules are found in a wide variety of conditions, and sometimes also in normal liver. Giant

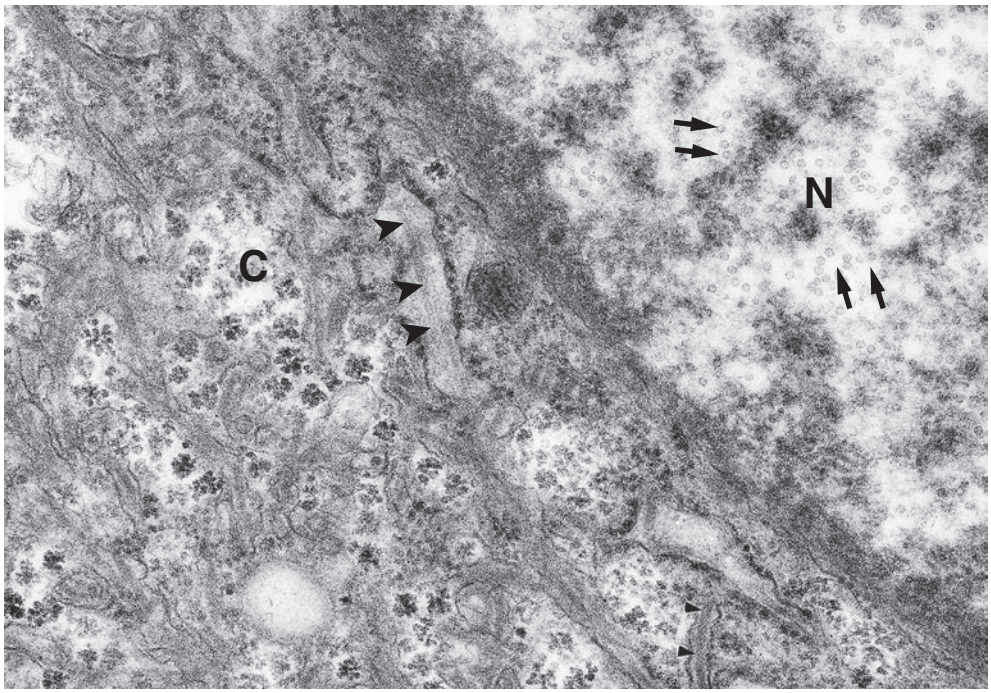

Fig. 17.7 Hepatocyte in hepatitis B surface material (HBsAg)-positive chronic hepatitis. In the nucleus (N) there are numerous core particles (arrows). The cytoplasm (C) contains irregularly shaped cisternae of the endoplasmic reticulum in which there are tubules (arrowheads), the morphological *in situ* counterpart of surface antigen. Glycogen rosettes are also visible in the cytoplasm at left. (Needle biopsy, lead citrate, 45,000×.)

mitochondria are seen most often in alcoholic liver disease[28] but are also found in non-alcoholic fatty liver disease[29] and other conditions.[30] Immunohistochemistry and immunoelectron microscopy can assist in their detection.[31] They are frequently found in patients with systemic sclerosis.[32] In the early stages of Wilson's disease mitochondria show variation in shape, increased electron density, widening of the spaces between membranes, vacuolation, enlargement of matrix granules and deposition of crystalline material[33,34] (**Fig. 17.8**). Three types of Wilsonian mitochondria are described which show intrafamilial concordance.[35] Abnormal, swollen and irregular mitochondria are found in hepatocytes in Reye's syndrome[36] and other microvesicular fat syndromes.[37] Highly irregular mitochondria are also seen in mitochondriopathies where there is respiratory chain dysfunction due to defects in mitochondrial DNA (mitochondrial depletion and deletion syndromes; **Fig. 17.9**). Affected neonates and infants may have liver failure and cholestasis in combination with neurological or neuromuscular disease[38–41] (neurohepatopathy) (**Ch. 13, Fig. 13.2**).

Endoplasmic reticulum

This is an important site of protein synthesis and transport. It also contains enzymes involved in drug and steroid metabolism. Morphologically, the ER is a cisternal membrane-bound system continuous with the nuclear envelope. It is the morphological counterpart of the microsomes. Two main types of ER can be recognised. The **rough-surfaced endoplasmic reticulum** is studded with ribosomes and is often arranged in a lamellar pattern. The **smooth-surfaced endoplasmic reticulum** lacks ribosomes and has a tubular or vesicular appearance.

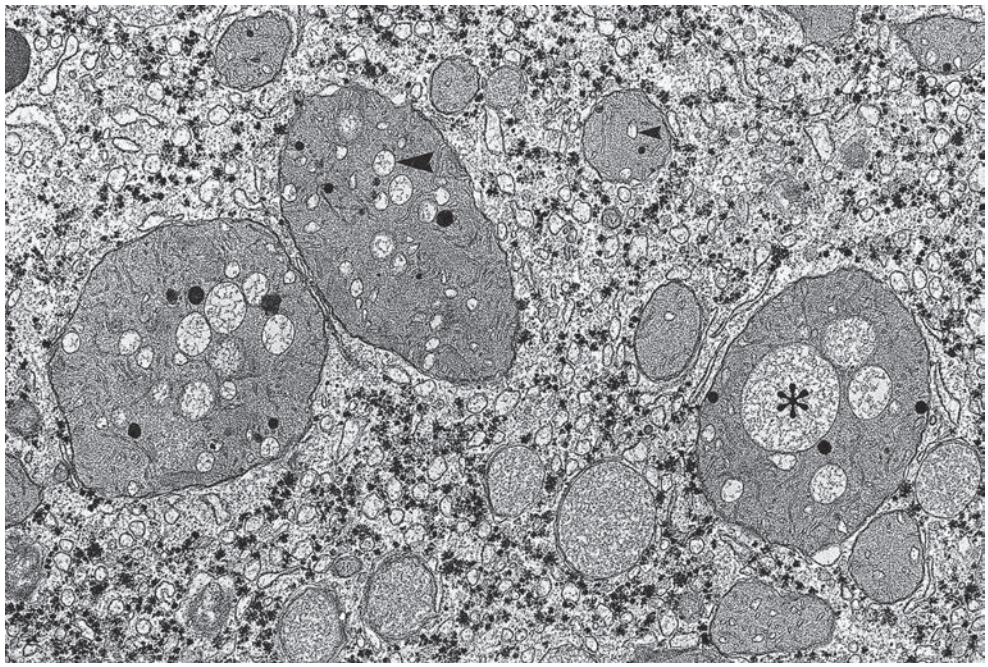

Fig. 17.8 Wilson's disease. Hepatocyte cytoplasm with mitochondria showing dilatation of intracristal spaces (arrowheads). Some are microcystic, and their contents are finely granular (*). Dense granules are prominent. (Ferrocyanide, 11,400×.) (Illustration kindly supplied by Professor MJ Phillips and Ms JS Patterson, Toronto, Canada.)

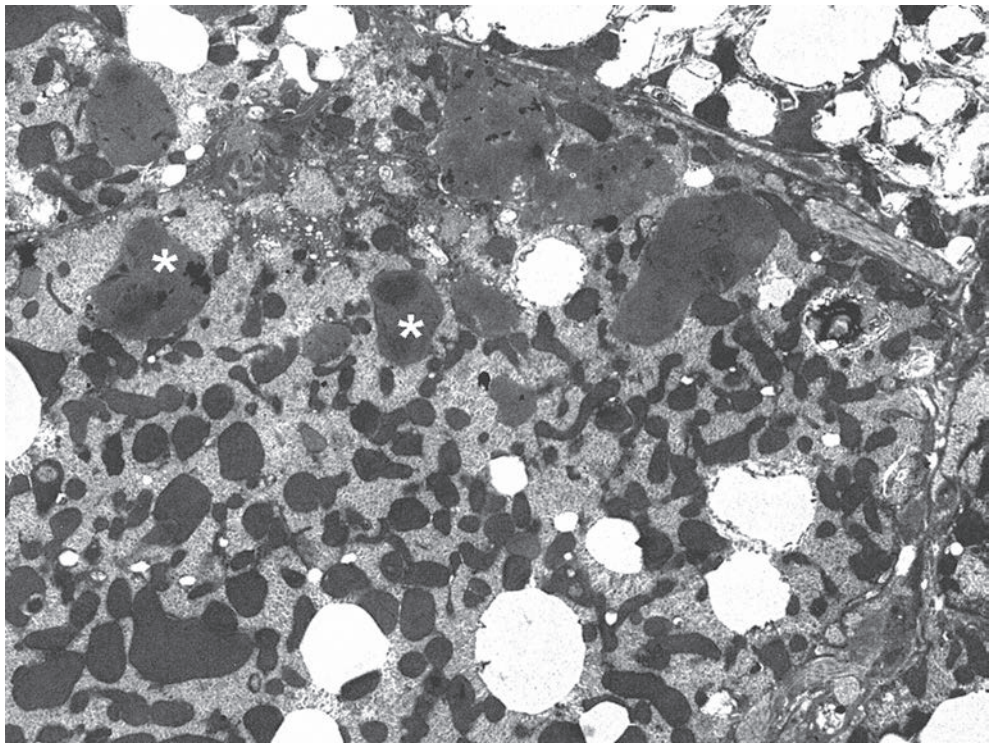

Fig. 17.9 Mitochondriopathy. Markedly pleomorphic mitochondria are present and show abnormal branching and tapering, marked enlargement and unusually large, dark osmiophilic matrix densities (mitochondria shown at *). Microvesicular fat vacuoles are also present. Genetic analysis of this infant with neurological deficits, liver failure and cholestasis demonstrated a mitochondrial DNA depletion syndrome. (Needle biopsy, osmium tetroxide.)

Structural Changes

Dilatation, degranulation, vesiculation and proliferation of the ER can be seen in many conditions. Some of these 'changes' are also influenced by the fixation procedure, making them difficult to evaluate. Their accurate quantification requires carefully controlled processing conditions and morphometric analysis. However, in α_1-antitrypsin deficiency, the dilatation of ER is striking, and finely granular material accumulates in the cisternae (**Fig. 17.10**). In chronic type B hepatitis the cisternae are also dilated and contain tubular structures representing the surface material of the hepatitis B virus (**Fig. 17.6**), and sometimes complete Dane particles.

Lysosomes

Lysosomes are organelles that carry many different lytic enzymes and are involved in the breakdown of proteins, carbohydrates and lipids. **Primary lysosomes** are small vesicles containing enzymes but not yet involved in catabolic processes. **Secondary lysosomes** are membrane-bound, often irregularly shaped electron-dense bodies in which the breakdown processes take place. When undigested residues accumulate and enzyme activity is diminished, the secondary lysosomes are called residual bodies. These are the lipofuscin granules. All types of secondary lysosomes tend to be concentrated around the bile canaliculi.

Structural changes

Lysosomes accumulate iron pigment in various forms of iron overload, including hereditary haemochromatosis.[42] They can be strikingly enlarged in inborn errors of metabolism, such as Niemann–Pick disease (**Fig. 17.2**) and type II glycogenosis, or show characteristic changes, such as in the Dubin–Johnson syndrome (**Fig. 17.3**). Lamellar and reticular inclusions are seen within them in acquired, drug-related phospholipidosis.[21–25]

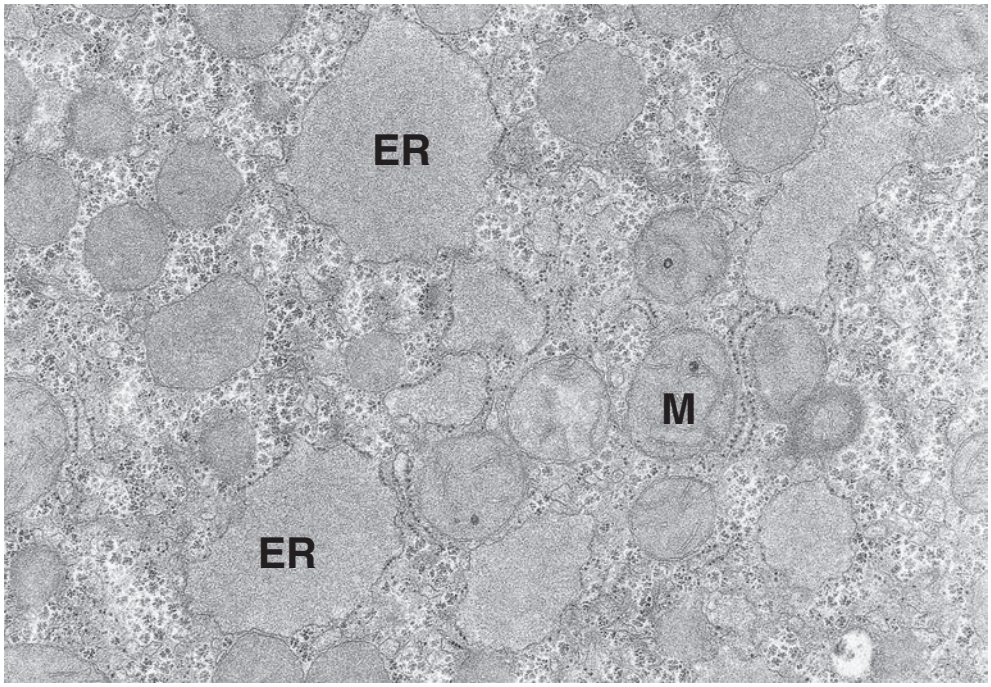

Fig. 17.10 Alpha$_1$-Antitrypsin deficiency. In this parenchymal cell the cisternae of the endoplasmic reticulum (ER) are dilated and filled with finely granular material. M, mitochondrion. (Needle biopsy, lead citrate; 16,000×.)

Peroxisomes

These are round or oval bodies with an even, granular matrix bounded by a single membrane. Human peroxisomes infrequently have a nucleoid, whereas this is often seen in other species. They are most numerous in perivenular hepatocytes. They contain numerous oxidative enzymes and are involved in β-oxidation of long-chain fatty acids and synthesis of bile acids and prostaglandins. Their catalase enzyme mediates conversion of peroxide to water.

Structural changes

Peroxisomes are absent in Zellweger's syndrome (cerebro–hepato–renal syndrome).[43] In alcoholic and drug hepatitis, the catalase content of peroxisomes is decreased[42] and they show irregular shapes.[43–45] Increased numbers of peroxisomes are seen in alcoholic and drug hepatitis[44] as well as in cirrhosis.[46]

Golgi apparatus

The Golgi apparatus is a membranous system involved in excretory functions of the cell. It contains enzymes such as glycosyl transferases and is involved in glycoprotein metabolism. Morphologically it is composed of small groups of flattened sacs with associated vesicles.

Structural changes

The appearance of the Golgi apparatus is influenced by fixation, and changes are therefore difficult to quantify, but dilatation is evident in regenerating liver and in hepatocellular carcinoma. Electron-dense liposomes accumulate in the system during the development of fatty liver.

Cell sap (cytosol)

The soluble portion of the cytoplasm (cell sap) contains variable amounts of glycogen, free ribosomes, microtubules, intermediate filaments and microfilaments. A few lipid droplets and scanty iron-containing granules are also seen.

Structural changes

Ferritin particles accumulate in iron storage disorders.[42] Fat droplets are numerous in fatty liver, but the amount of fat varies greatly with the patient's state of nutrition. Core particles of hepatitis B virus can be identified in the cytoplasm in many cases of chronic type B hepatitis. Cytoplasmic crystalline inclusions are seen both in normal and in diseased livers. In alcoholic hepatitis, the Mallory–Denk bodies found in ballooned hepatocytes are composed of accumulations of cytokeratin and other proteins in the form of filaments (**Fig. 17.11**).

Bile canaliculus

The bile canaliculus measures approximately 0.75 μm and is formed by membranes of several contiguous hepatocytes which are joined by tight junctions.[47] Surface microvilli covered with a thin glycoprotein coat project into the canalicular lumen. Actin filaments are present within the microvilli and extend downward into a pericanalicular web also composed of actin, functioning in canalicular contraction.

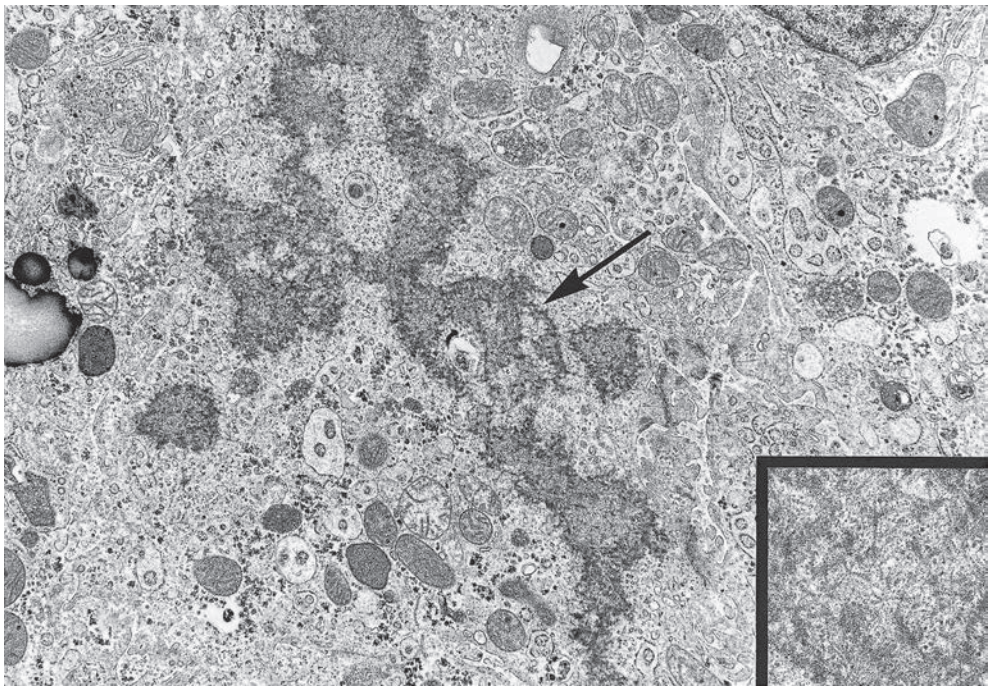

Fig. 17.11 Mallory–Denk bodies. Irregular electron-dense material (arrow) is seen in the cytoplasm of a hepatocyte. The fibrillar nature of the material is evident at the higher magnification shown in the inset. (Needle biopsy, uranyl acetate and lead citrate; 8600×; inset: 27,000×.)

Structural changes

Alterations in the bile canaliculus are similar in many forms of cholestasis. Loss of microvilli, formation of surface membrane blebs and disorganisation of the pericanalicular actin filament web are common features. In the cholestasis related to preservation injury after liver transplantation, for example, ischaemia and reperfusion injury result in canalicular dilatation, loss of microvilli and compaction of actin filaments.[48] Intracanalicular bile appears as electron-dense filamentous material. Coarsely granular canalicular bile (Byler bile) is a characteristic of progressive familial intrahepatic cholestasis type 1 (Byler disease) seen in Amish children[49] (**Fig. 17.12**).

Glycogen

Glycogen particles are normally distributed throughout the cytoplasm among other organelles, but are often near the smooth ER. Monoparticulate glycogen (beta) particles are deeply osmiophilic 7–18-nm polygonal granules. However, the most common type of glycogen granules seen in normal hepatocytes are 200-nm glycogen rosettes (alpha particles) which consist of aggregates of monoparticulate granules (**Fig. 17.7**).

Structural changes

Glycogen storage diseases[50] (glycogenoses) and certain cases of poorly controlled diabetes ('glycogenic hepatopathy'; **Ch. 7**) show excessive cytoplasmic glycogen granules within distended hepatocytes. Pools of monoparticulate glycogen displace mitochondria and other organelles towards the cell membrane (**Fig. 17.13**), resulting in the light microscopic appearance of thickened, plant-like hepatocyte membranes. In type II glycogenosis, intralysosomal glycogen deposits are present, while intranuclear glycogen is a feature of glycogenosis type Ia (as well as diabetes, insulin resistance, childhood and Wilson's disease).

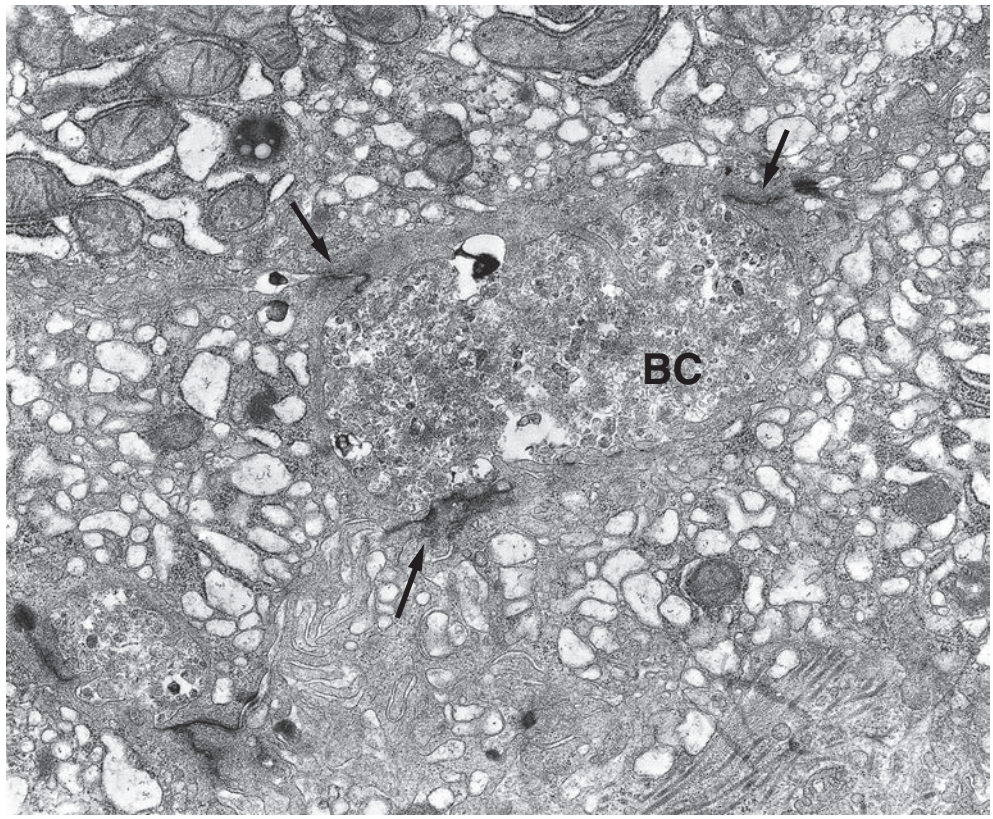

Fig. 17.12 Progressive familial intrahepatic cholestasis, type 1 (PFIC-1). The dilated bile canaliculus (BC) contains coarsely granular bile (Byler bile), a feature associated with cholestasis in Amish children. The canaliculus is delimited by several junctional complexes (arrows) and has a reduced number of microvilli. (Needle biopsy, lead citrate; 24,475×.) (Illustration kindly provided by Dr AS Knisely, Galveston, Texas, USA.)

Ground-glass-like cytoplasmic inclusions of abnormal glycogen granules in hepatocytes may be seen in adult polyglucosan body disease,[51] in Lafora disease[52] (myoclonus epilepsy) and in certain recipients of organ transplants.[53]

Kupffer cell

The Kupffer cell has an irregular outline, with many finger-like protrusions of the cell surface by which it anchors to endothelial cells. It is rich in phagocytic vacuoles (phagosomes), lysosomes and mitochondria, while the ER is only moderately well developed. The nucleus is irregular in shape, with a tendency for the chromatin to be concentrated at the nuclear periphery.

Structural changes

Hypertrophied Kupffer cells can be seen in all conditions of parenchymal cell destruction (e.g. hepatitis) and in pigment overload (e.g. cholestasis, siderosis). Many storage disorders affect the Kupffer cells; in Niemann–Pick disease, for example, both Kupffer cells and hepatocytes are enlarged and filled with vacuoles containing accumulated sphingomyelin (**Fig. 17.2**).

Endothelial cell

The endothelial cell is a flattened cell with a smooth surface, showing small fenestrae organised into sieve plates which provide direct communication between the sinusoidal lumen and the space of Disse.[54] Fenestrae show open and multifolded labyrinth-like

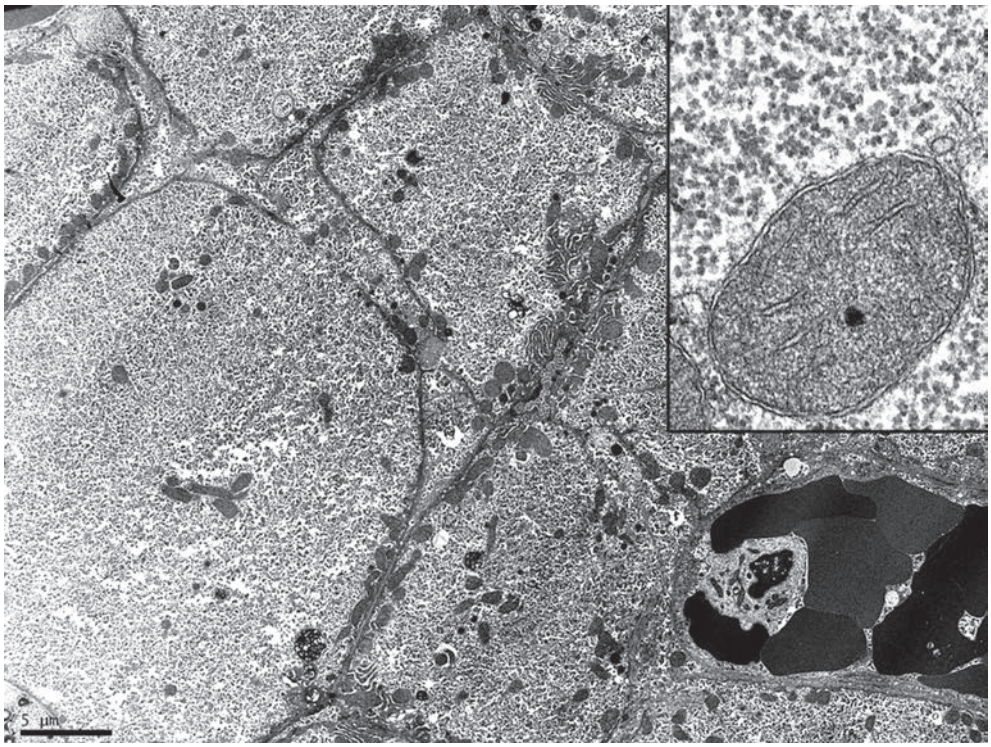

Fig. 17.13 Glycogen storage disease. Several hepatocytes are seen in this field, each of which shows pools of monoparticulate glycogen within the cytoplasm and displacement of most mitochondria and other organelles towards the cell membranes. This type of glycogenosis is seen in several types of glycogen storage disease, including type I (von Gierke's disease). Inset: High magnification shows monoparticulate glycogen particles (each particle is approximately 7–18 nm in diameter), with a normal-appearing mitochondrion nearby for size comparison. (Needle biopsy, osmium tetroxide.)

configurations.[55] The cytoplasmic volume is relatively small. Many micropinocytotic vesicles can be seen beneath the plasma membrane.

Structural changes

In hepatitis and other conditions, endothelial cells undergo several changes, including the accumulation of iron-rich siderosomes and the formation of basement membrane material on the aspect of the cells facing the space of Disse.[56] In patients with chronic viral hepatitis and acquired immunodeficiency syndrome (AIDS), **tubuloreticular structures** and **cylindrical confronting cisternae** develop within the rough ER of endothelial cells and sometimes within Kupffer cells, stellate cells (discussed later) and lymphocytes.[57,58] Tubuloreticular structures are reticular aggregates of branching tubules within the cisternae of the ER and sometimes the perinuclear envelope. Cylindrical confronting cisternae are cylinders of fused membranous lamellae derived from two or more cisternae of ER, one inside the other. They appear to be a result of increased endogenous levels of α- and β-interferon. Membrane-bound dense bodies, seen on light microscopy as diastase–periodic acid–Schiff (PAS)-positive cytoplasmic granules, are sometimes present in chronic hepatitis B and C and autoimmune hepatitis[59] (**see Fig. 9.12**).

Hepatic stellate cell (Ito cell)

The hepatic stellate cell (HSC), previously known as the Ito cell, fat-storing cell, perisinusoidal cell or lipocyte, is a major storage site for vitamin A. In liver injury, it becomes

a transitional cell or myofibroblast-like cell capable of synthesising collagen types I, III and IV as well as laminin.[60,61] Stellate cells are located within the space of Disse (**see Fig. 7.8**) and have conspicuous rough ER, a large Golgi apparatus and large lipid droplets containing vitamin A. HSCs are considered the chief hepatic cell responsible for scarring and fibrosis of the liver in a variety of disorders, including cirrhosis.[62] In alcoholic liver disease, hypervitaminosis A and methotrexate toxicity, stellate cells undergo hyperplasia and are associated with increased collagen fibres within the space of Disse. Multivesicular stellate cells with numerous lipid droplets have been reported in primary biliary cirrhosis.[63]

Pit cell (large granular lymphocyte)

This cell is located within the sinusoidal lumen, preferentially in the periportal region compared to acinar zone 3.[64] Its surface uropodia and pseudopodia are often in close contact with endothelial cells or Kupffer cells. The nucleus is dense, eccentrically located in the cell and indented. The cell's name derives from its characteristic electron-dense, membrane-bound granules of cytotoxic enzymes which resemble 'pits' or pips in fruit. The cytoplasm contains profiles of rough ER, a well-developed Golgi apparatus, centrioles and occasional rod-cored vesicles. Pit cells function as natural killer cells and have been identified in autoimmune hepatitis and in increased numbers in livers with malignant tumours.[65]

Extracellular vesicles

Exocytosis of membrane-bound extracellular vesicles (EVs) from the sinusoidal surfaces of hepatocytes has drawn recent interest because of their 'cargo' and their potential value in diagnosis, therapy and prognosis.[66,67] EVs vary in size from the smallest, multivesicular body-derived *exosome* (40–100 nm) to the larger *microvesicle* (0.1–1 μm), large *apoptotic body* (1–4 μm) and potentially largest, hepatocellular carcinoma-derived *oncosome*. EV cargo includes micro-RNA, heat shock proteins, hepatitis viruses and lymphocyte markers.[66] EVs can be visualised by TEM and by cryo-electron microscopy, the latter technique allowing reconstruction of the surface topography of these particles. Joachim Frank (Columbia University) was awarded the 2017 Nobel Prize for his decades-long contributions to the field of cryo-electron microscopy.[68] EV biology has been utilised to understand the pathogenesis of a number of liver diseases, including non-alcoholic fatty liver disease, hepatocellular carcinoma, and, recently, the mechanism of transmission of hepatitis E virus (HEV).[69]

Immunoelectron microscopy

The principles employed in immunohistochemical staining of liver biopsy sections for light microscopy (see the 'Immunohistochemistry' section, later) can be adapted for use in electron microscopy.[70] Following fixation of the specimen in a mixture of glutaraldehyde and paraformaldehyde, the tissue is treated with borohydride, cryoprotected and frozen for storage. Thick sections of 20–40 μm are later cut from the thawed samples and stained by either a direct or indirect immunoperoxidase method.[71] The stained sections are then post-fixed in osmium tetroxide, dehydrated and embedded in Epon. Under the electron microscope, electron-dense immunoreactive material is seen at the site of the target antigen.

Availability of a wide variety of monoclonal and polyclonal antibodies to tissue antigens and receptors has greatly expanded investigations of interactions of hepatocytes with immune cells and with the extracellular matrix. Intercellular adhesion molecules, histocompatibility antigens and interferon receptors are among the potential list of antigens that can be studied by immunoelectron microscopy.[72–74] An example of this technique is shown in **Fig. 17.14**, which demonstrates the upregulation of the type A receptor for tumour necrosis factor on hepatocyte membranes in a patient with chronic hepatitis B.[75]

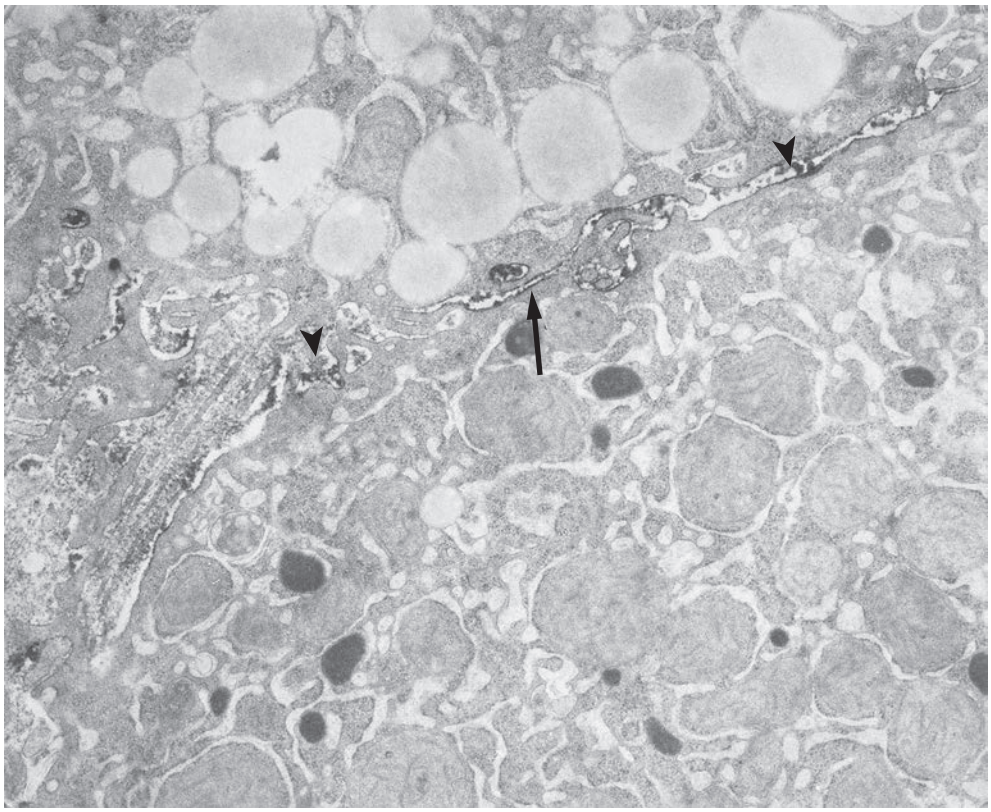

Fig. 17.14 Type A receptor for tumour necrosis factor (TNF). A case of hepatitis B virus–positive chronic hepatitis stained with monoclonal antibody Utr-1 (directed against type A receptor of TNF) shows positive staining on the membranes of two adjacent hepatocytes in a discontinuous pattern (arrow) and in the intercellular space (arrowheads). (Immunoelectron microscopy, 18,400×.) (Illustration kindly provided by Drs VJ Desmet, R Volpes, J Van den Oord and R De Vos, Leuven, The Netherlands.)

Scanning electron microscopy

The three-dimensional structure of the liver can be assessed by scanning electron microscopy of specially prepared tissues,[76] or even of sections from paraffin blocks[77] (**Fig. 17.15**). X-ray microanalysis may be combined with the scanning technique and is useful in elemental analysis. Laboratories with scanning electron microscopes are best equipped to provide details on appropriate tissue fixation, critical point drying and coating of specimens with gold or platinum. Scanning electron microscopy is useful in examining bile ducts[78] and resin casts of hepatic vasculature.[79–82]

Immunohistochemistry

Immunohistochemical techniques are widely available in pathology laboratories and the methods for both immunoperoxidase stains and immunofluorescence microscopy are covered in standard textbooks.[83] The utility of immunostains for specific keratins in the catalogue of Moll[84] and, particularly, of cytokeratins 7 and 20 in determining the histogenesis

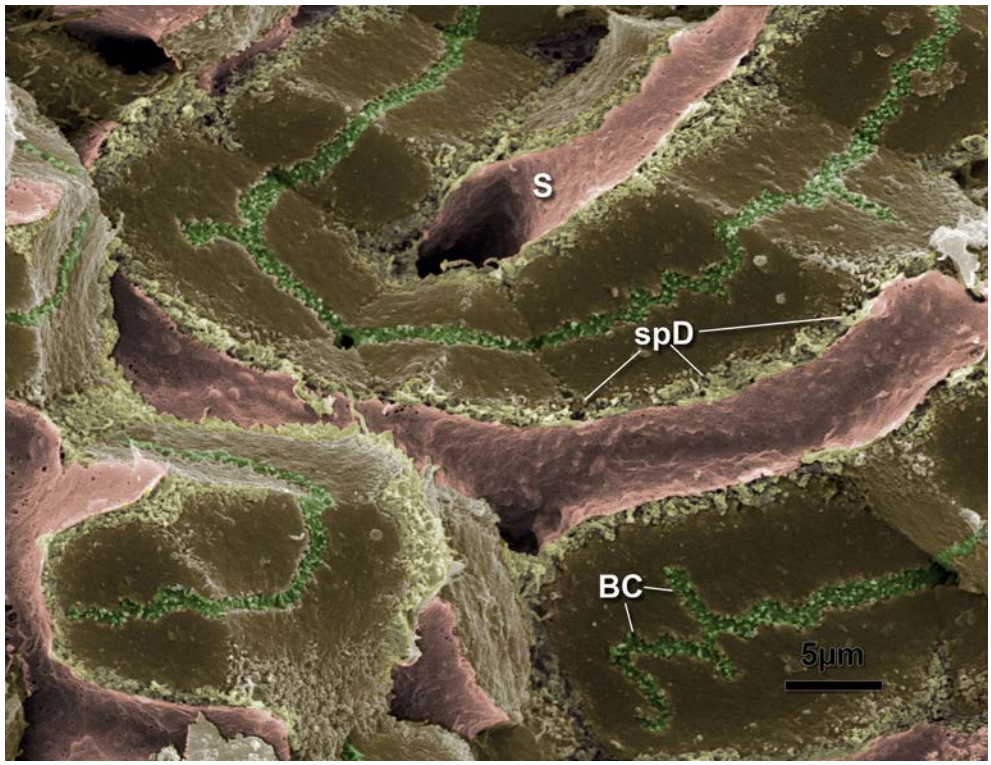

Fig. 17.15 Colourised scanning electron micrograph of liver. Sinusoids (S) (light pink) course between the hepatic cords (green). The network of bile canaliculi (BC) is well demonstrated. Note the narrow space of Disse (spD) between the sinusoidal endothelial lining cells and the surfaces of hepatocytes. (Micrograph kindly provided by Jackie Lewin, UCL Medical School, London, UK.)

of tumours[85] is widely recognised and is also important in the evaluation of hepatic neoplasms. Specific immunostains for the diagnosis of hepatocellular carcinoma and other hepatobiliary tumours are discussed in detail in Chapter 11 (**see Fig. 11.18**). Demonstration of hepatitis B viral antigens in the context of chronic hepatitis and cirrhosis is addressed in Chapter 9. Other viruses such as cytomegalovirus can also be studied immunohistochemically (e.g. post–liver transplantation).[86] Cytokeratin 7 immunostain has special value for the identification of native bile ducts, the ductular reaction and hepatic progenitor cells and their derivatives, and is therefore discussed in many sections of this book. The use of ubiquitin immunostain for Mallory–Denk bodies and combined cytokeratin 8 and 18 immunohistochemistry for damaged and ballooned hepatocytes in steatohepatitis is outlined in Chapter 7. There is a large and growing menu of immunohistochemical stains for potential use in day-to-day liver biopsy practice as well as for research studies on liver pathobiology. These are mentioned throughout the course of this book and can be updated by consulting PubMed and other internet resources.

Gene array, gene sequencing and molecular analysis

The recent elucidation of the human genome and the expanded availability of many techniques for analysing gene expression patterns and signatures, as well as mutational sequences, have had an enormous impact on basic science and clinical studies in hepatology. Various types of liver specimens can be used for genomic and molecular analysis, including fresh tissue, formalin-fixed and paraffin-embedded tissue,[87] touch imprints[88] and archival tissue blocks, provided that their DNA and RNA are sufficiently well preserved. The liver transcriptosome expresses some 25%–40% of the 39,000 genes in the human

genome[89] and their expression patterns and alterations can be studied by gene microarray analysis and other methods. Combining techniques, such as *in situ* hybridisation with laser capture microdissection and polymerase chain reaction, can increase the sensitivity of the analysis, according to certain studies.[90] Genome-wide studies identify gene signatures that can be implicated in the pathogenesis of diverse liver diseases such as chronic hepatitis C and non-alcoholic fatty liver disease.[91] Sanger sequencing[92] and 'next-gen' (deep) sequencing methods can now be utilised to characterise the types of gene mutations present in specific tumours as a component of personalised genomic medicine and to provide targeted therapy.[93] An example of this type of sequencing analysis, in this instance for *KRAS* mutation, is shown in **Fig. 17.16**.

Other Techniques

Special investigations, such as confocal microscopy, *in situ* hybridisation, polymerase chain reaction and laser capture microdissection, are now widely used in pathology departments

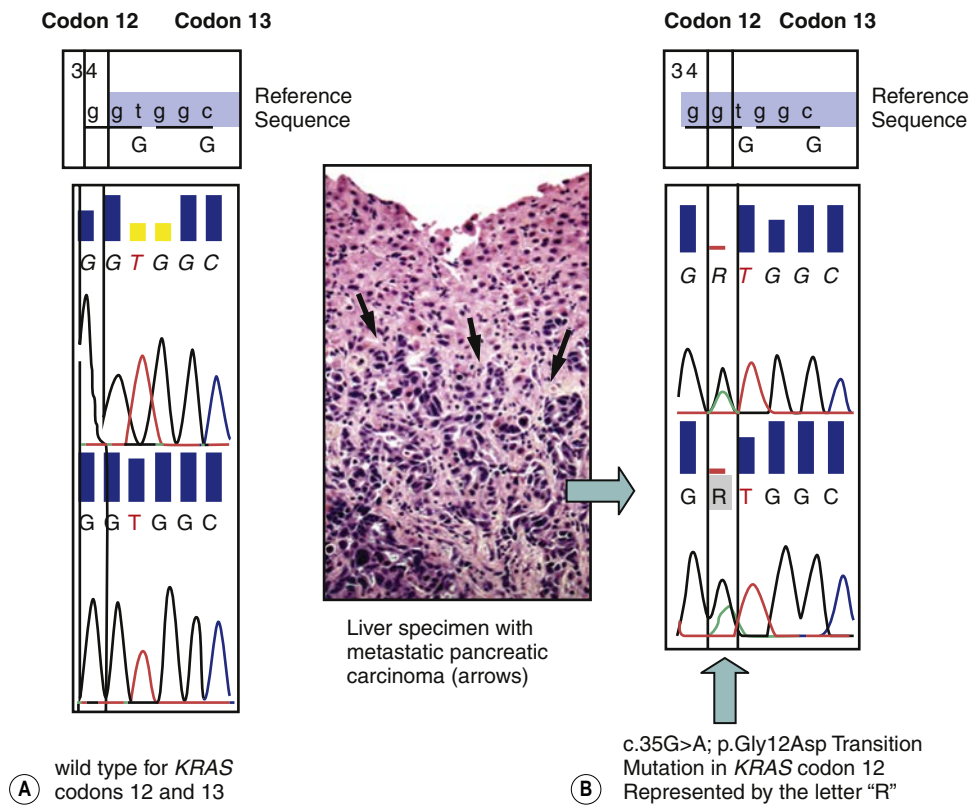

Fig. 17.16 Direct DNA-PCR di-deoxyterminator sequencing of codons 12 and 13 of the *KRAS* gene on paraffin-embedded, microdissected tissue from selected liver metastases. A: This wild-type *KRAS* sequence of codons 12 and 13 was found in a poorly differentiated colorectal adenocarcinoma metastasis to segment 4 of the liver in a 60-year-old female with a history of colorectal adenocarcinoma of the left colon and a previous history of liver metastases. **B:** This tumour had a mutant *KRAS* sequence which demonstrated the following transition mutation (at yellow arrow) affecting base pair 35 in codon 12: c.35G>A; p.Gly12Asp. This mutation was found in a poorly differentiated adenocarcinoma liver metastasis in a 62-year-old male patient with a history of pancreatic adenocarcinoma (a portion of the resected tumour is shown to the left of the *KRAS* sequence).

and other biomedical venues and can also be implemented for evaluating liver specimens. These procedures require specific fixation and other procedural conditions, as indicated in **Table 17.1**. Techniques such as cryo-electron microscopy and atomic force microscopy (AFM)[94,95] allow structural observations to be made under more physiological conditions because the specimens are processed differently and spared the damaging treatments that occur with the usual fixation, coating and electron beam exposure. These techniques are complex, however, requiring special equipment and software, and are therefore more suitable at present for basic research than for diagnosis. The reader is encouraged to consult the numerous publications available through PubMed, other internet sources and textbooks for additional details on methodology and potential areas of investigation.

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Glossary

Note: Words in *italics* are defined elsewhere in the glossary.

Acidophil body (Figs 6.2 and 16.16) A *hepatocyte* which has undergone apoptosis; now often referred to as an apoptotic body. See also *Councilman bodies*.

Acinus (Fig. 3.1) An anatomical unit based on blood supply, its three parenchymal zones containing successively less oxygenated blood. Zone 1 is nearest to the terminal portal vessels in a small portal tract.

Activity (Figs 9.7 and 9.8) In histological terms, an expression of the degree of hepatocellular damage and associated inflammation. Especially used in chronic hepatitis and cirrhosis, in which it forms the basis of *grading*.

Apoptosis (Figs 6.2 and 16.16) Shrinkage and fragmentation of cells, seen in the liver mainly in the form of densely stained rounded structures derived from hepatocytes but lying free outside the *liver-cell plates*.

Autoimmune hepatitis A form of hepatitis associated with high titres of autoantibodies in serum. Usually responds to immunosuppressive therapy.

Ballooning degeneration Swelling and rounding of hepatocytes, with loss of their normal polygonal shape. Different forms of ballooning are seen in viral hepatitis (Fig. 6.2) and *steatohepatitis* (Fig. 7.10C).

Bile canaliculus (Figs. 5.2 and 5.3) The tubular space formed between the biliary poles of two or three *hepatocytes*, or more in diseased liver. The canaliculus has no separate epithelial lining of its own.

Bile duct (Fig. 3.2) The smallest ducts, the interlobular bile ducts, are centrally located in small portal tracts and are usually accompanied by blood vessels. In practice they are sometimes difficult to distinguish from *bile ductules*, the transition being gradual.

Bile duct plate (Fig. 4.16) The foetal periportal structure containing hepatoblast-derived epithelium that gives rise to the intrahepatic bile ducts by the process of tubulogenesis.

Bile ductule and canal of Hering (Fig. 3.3) At the portal–parenchymal interface the canalicular system drains into the *canals of Hering* which are partly lined by hepatocytes and partly by biliary epithelial cells (*cholangiocytes*). These in turn connect with bile ductules, fully lined by biliary epithelium.

Bile extravasate (Fig. 5.9) Leakage of bile from a duct into the connective tissue of the portal tract, occasionally seen in large bile-duct obstruction.

Bile infarct (Fig. 5.4) An area of liver-cell death in a cholestatic liver; often periportal, whereas canalicular *cholestasis* is mainly perivenular. Bile staining is variable and may be absent. Bile infarcts are easily mistaken for accumulations of foamy macrophages.

Bile lake An accumulation of bile outside a *liver-cell plate*.

Bile thrombus (Fig. 5.2) Synonymous with bile plug: the accumulation of visible bile in a *bile canaliculus*.

Bilirubinostasis A term sometimes used for histological cholestasis.

Bridging fibrosis (Fig. 7.21) Linking of portal tracts and/or efferent venules by fibrous tissue.

Bridging necrosis (Fig. 6.9) Confluent hepatocellular necrosis and *collapse* linking vascular structures; usually and preferably confined to linking of portal tracts to efferent venules.

Canals of Hering (Fig. 3.3) Structures lined partly by *hepatocytes* and partly by bile ductular epithelium. They are a probable site of *progenitor cells*.

Central perivenulitis (Fig. 16.21) A feature of liver transplant rejection in which efferent venules are targeted by lymphocytes and other effector immune cells. Dropout and *apoptosis* of perivenular *hepatocytes* and focal congestion are also frequently present. This common manifestation of paediatric allograft rejection is sometimes present in combination with classical portal tract rejection changes and occasionally is seen late (>1 year after transplantation) as the isolated expression of rejection.

Ceroid pigment (Fig. 6.5) Brown pigment in macrophages, found after hepatocellular injury; rich in oxidised lipids and PAS-positive after diastase digestion. Distinct from *lipofuscin*.

Cholangiocyte Epithelial cell of the biliary tract.

Cholate stasis (Fig. 5.10) A term sometimes used for chronic *cholestasis*, on the assumption that the hepatocellular changes result from the accumulation of toxic bile salts. Also known as *precholestasis* or *pseudoxanthomatous change*.

Cholestasis (Fig. 5.2) In morphological terms, *bilirubinostasis* or visible bile in a section of liver. Also defined as failure of bile to reach the duodenum and biochemically as a type of jaundice with dark urine, pale stools, conjugated hyperbilirubinaemia and raised serum alkaline phosphatase level.

Cirrhosis The transformation of the normal hepatic architecture into nodules separated by *fibrosis*.

Collapse (Fig. 4.8) Condensation of pre-existing reticulin framework as a result of necrosis. May be followed by *fibrosis*.

Confluent necrosis (Fig. 8.4) Death of groups of adjacent *hepatocytes*.

Councilman bodies *Hepatocytes* which have undergone *apoptosis*. The term is best restricted to yellow fever, the disease in which they were described by Dr Councilman.

Disse space (Fig. 17.4) The space between the sinusoidal endothelium and *hepatocytes*; contents include extracellular matrix and *hepatic stellate cells*.

Ductopenia (Figs 13.6 and 16.10) Loss of significant numbers of interlobular *bile ducts*. Causes include rejection of liver grafts, graft-versus-host disease, primary biliary cirrhosis, primary sclerosing cholangitis and drug injury. Diseases characterised by ductopenia are known as *vanishing bile duct syndromes*.

Ductular proliferation Use of this term is discouraged for the reason given in the next definition.

Ductular reaction (Fig. 4.13) A reaction of ductular phenotype, seen as an increase in ductular structures. This may be the result of proliferation of pre-existing ductules, but the new structures could also arise from biliary metaplasia of *hepatocytes* or from transformation of *progenitor cells*.

Dysmetabolic hepatic iron overload (DHIO) (Fig. 7.12) *Siderosis* of *Kupffer cells* and/or *hepatocytes* due to insulin resistance and its effects on iron homeostasis. Most often evident histologically as iron overload in the setting of macrovesicular *steatosis* in non-alcoholic fatty liver disease.

Dysplasia (Figs 10.8 and 10.9) A change in the size, nucleus-to-cytoplasm ratio and/or nuclear appearances of *hepatocytes*, usually in chronic hepatitis and *cirrhosis*. Large-cell and small-cell types are described. Also known as large- and small-cell change.

Fat-storing cells *Hepatic stellate cells*.

Fatty liver disease Includes both *steatosis* and *steatohepatitis*, as in alcoholic fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD).

Feathery degeneration (Fig. 5.3) A type of liver-cell injury in *cholestasis*, attributed to toxic effects of bile salts. Affected *hepatocytes*, often single cells lying within normal *parenchyma*, are swollen and have pale-staining feathery cytoplasm.

Fibrosis Formation of new collagen fibres. It may follow *collapse* of pre-existing connective tissue framework or arise *de novo*.

Focal necrosis (Fig. 9.8) Death of *hepatocytes*, singly or in small groups. Because of the rapid disappearance of the dead cells, focal necrosis is usually recognised by the presence of inflammatory cells and by a break in continuity of a *liver-cell plate* rather than by the presence of necrotic tissue.

Follicle See *lymphoid follicle*.

Glycogen vacuolation See *nuclear vacuolation*.

Grading Semi-quantitative scoring of the various processes comprising hepatocellular damage and inflammation, usually in chronic hepatitis. Numerical assessment of histological activity.

Granuloma (Fig. 15.1) A focal accumulation of epithelioid cells, which are modified macrophages with abundant cytoplasm and often curved, elongated nuclei. To be distinguished from simple accumulations of macrophages.

Ground-glass hepatocytes (Fig. 9.13) *Hepatocytes* with a well-defined, lightly eosinophilic homogeneous area occupying much of the cytoplasm. The most common form is seen in the livers of patients infected with the hepatitis B virus.

Haemochromatosis (See also *siderosis.*) A condition in which hepatic *fibrosis* and *cirrhosis* ultimately develop as a result of iron overload. The common form, hereditary haemochromatosis, is usually the result of mutations of the *HFE* gene on chromosome 6.

Hepatic stellate cells (Figs 7.6 and 17.4) Cells containing vacuoles rich in vitamin A, lying within the *Disse space*. In pathological conditions, they are able to transform into myofibroblasts and produce extracellular matrix components. Previously used synonyms include *fat-storing cells*, *Ito cells*, *lipocytes*, *parasinusoidal cells* and *perisinusoidal cells*.

Hepatocytes Liver cells.

Interface hepatitis (Figs 9.3 and 9.4) Death of *hepatocytes* at the interface of connective tissue and *parenchyma* in chronic liver disease, accompanied by inflammatory-cell infiltration. Characteristic of chronic hepatitis and synonymous with the older term *piecemeal necrosis*.

Ito cells *Hepatic stellate cells*.

Kupffer cells The resident macrophages of the liver, straddling the sinusoidal lumens.

Limiting plate The layer of *hepatocytes* next to a portal tract.

Lipocytes *Hepatic stellate cells*.

Lipofuscin (Fig. 3.6) Pigmented granular material in *hepatocytes*, of lysosomal origin and most abundant at the biliary poles of the cells. Found in normal liver in greatly varying amounts.

Liver-cell plates (Fig. 3.5) Interconnecting walls of *hepatocytes*, one cell thick in adults. Thicker plates are found in children and in regenerating liver.

Lobular activity (Fig. 9.8) Inflammation and hepatocellular damage deep within the lobules, in contrast to *interface hepatitis*.

Lobule (Fig. 3.1) An anatomical unit with an efferent (centrilobular) vein at its centre and portal tracts peripherally.

Lupoid hepatitis An old term for *autoimmune hepatitis*, no longer in use.

Lymphoid follicle (Figs 9.16 and 9.17) A structured accumulation of lymphocytes resembling the follicles of normal lymph nodes.

Mallory bodies (Figs 7.17 and 17.10) Irregular, dense cytoplasmic inclusions with a cytokeratin component, often in the form of strands or garlands. Electron microscopy reveals a filamentous structure.

Massive necrosis (Figs 4.13D and 6.12) *Multilobular necrosis* involving a substantial part of the whole liver. This usually leads to severe liver insufficiency.

Metabolic syndrome The association of insulin resistance with central (truncal) obesity, diabetes mellitus, hyperlipidaemia and systemic arterial hypertension. Non-alcoholic fatty liver disease (NAFLD) is considered the hepatic expression of the metabolic syndrome.

Multilobular necrosis (Figs 4.13D and 6.12) Confluent necrosis involving the whole of several adjacent lobules. The clinical effects are variable, depending on the extent of the lesion.

Non-alcoholic steatohepatitis (NASH) (Fig. 7.22) A form of hepatitis resembling alcoholic *steatohepatitis* but associated with other causes such as obesity, diabetes or drugs.

Nuclear vacuolation (Fig. 7.13) Empty *hepatocyte* nuclei in paraffin sections. May be due to glycogen accumulation, lipid or invagination of cytoplasm. Glycogen nuclei, common in the young, the obese and the diabetic, are typically enlarged and have prominent nuclear membranes. The glycogen may be demonstrable histochemically but is often lost during processing.

Panacinar necrosis (Fig. 6.12) Necrosis of an entire *acinus*.

Panlobular necrosis (Fig. 6.12) Necrosis of an entire *lobule*.

Parasinusoidal cells *Hepatic stellate cells*.

Parenchyma The specialised tissue of the liver, as opposed to the connective tissue. Often used loosely to describe the contents of the *lobules* as opposed to the portal tracts.

Periportal The part of the hepatic *lobule* or *acinus* next to a small portal tract.

Perisinusoidal cells *Hepatic stellate cells*.

Piecemeal necrosis *Interface hepatitis* is now often used as the term for this process, because it almost certainly involves *apoptosis* rather than, or as well as, necrosis.

Polyploidy (Fig. 3.9) The coexistence of different classes of nuclei containing multiple sets of chromosomes (e.g. quadriploid, octaploid); a normal state in adult human liver.

Portal tracts (Figs. 3.2 and 4.2) The connective tissue units at the periphery of lobules which contain *portal triads* (intrahepatic bile ducts and branches of the hepatic artery and portal vein).

Portal triad (Fig. 3.2) The triad of artery, vein and *bile duct* present in most portal tracts.

Precholestasis (Fig. 5.10) See *cholate stasis*.

Progenitor cell A partly committed cell capable of producing a range of specialised cell types. In the liver, progenitor cells are probably located in *bile ductules* or *canals of Hering*. See also *stem cell*.

Pseudoacini *Rosettes*.

Pseudoxanthomatous change (Fig. 5.10) See *cholate stasis*.

Regeneration (Fig. 10.6) Loosely used to describe hepatocellular hyperplasia following injury or loss. Not easily recognised in conventional sections because of low mitotic rate; characterised by increase in the thickness of the cell plates.

Rosettes (Figs 4.11, 9.9 and 9.18) In liver pathology this term refers to a change of the normal plate pattern of *hepatocytes* to glandular structures formed by several *hepatocytes*. Different types of rosette formation are seen in *cholestasis* and in chronic hepatitis.

Septa (Figs 10.15 and 10.16) Walls of fibrous tissue, seen in two-dimensional sections as lines or bands. Septa may be formed by *collapse* (passive septa), by new fibre formation ('active septa') or by both.

Siderosis The presence of stainable iron in any component of liver tissue. The many causes of siderosis include several diseases under the heading of *haemochromatosis*, in which progressive

iron accumulation leads to *fibrosis* and *cirrhosis*. However, at an early stage of hereditary *haemochromatosis* there is iron deposition without *fibrosis*.

Sinusoidal obstruction syndrome (Fig. 16.22) Circulatory obstruction within hepatic sinusoids and efferent venules following endothelial damage due to myeloablative chemotherapy or exposure to toxins such as pyrrolizine alkaloids. The term is often used as an alternative to veno-occlusive disease.

Spotty necrosis Widespread but patchy hepatocellular necrosis, typical of acute hepatitis.

Staging The semi-quantitative assessment of structural changes including *fibrosis* and *cirrhosis*.

Steatohepatitis (Fig. 7.16) A form of hepatitis characterised by *steatosis*, hepatocellular *ballooning*, *Mallory bodies* and pericellular *fibrosis*.

Steatosis (Figs 7.1 and 7.2) The accumulation of excess lipid in *hepatocytes*.

Stellate cells See *hepatic stellate cells*.

Stem cell A self-renewing cell with the potential to give rise to a variety of cells, including *progenitor cells*.

Tubulogenesis The process of intrahepatic bile duct development beginning at 8 weeks of gestation whereby cells of the periportal ductal plate evolve into nascent tubules and native intrahepatic bile ducts.

Vanishing bile duct syndromes Disorders characterised by loss of *bile ducts* leading to *ductopenia* (paucity of ducts) with consequent *cholestasis*.

Index

A

Abernethy malformation, 270, 271f
Aberrant biliary keratin (keratin 7), in heart failure, 276
Abnormal macrophage pigment, 56
Aceruloplasminaemia, 331
Acetaminophen, 141, 141f, 143f
Acidophil body. *see* Apoptotic body
Acinus, 20, 21f
Acquired immune deficiency syndrome (AIDS), 344–349
  antiretroviral therapy for, 347f
  cholangiopathy, 347
  drug-related hepatotoxicity, 346
  hepatobiliary lesions in, 346t
  Mycobacterium avium–intracellulare, 346–347, 348f
  opportunistic infections and infestations, 346–347
  peliosis hepatis, 347–348
Activity, 1
  definition, 201
Acute cholangitis, 67f
Acute cholestasis, 44
Acute drug-induced hepatitis, 142–143, 142f–143f, 143b
Acute fatty liver of pregnancy (AFLP), 366–367
Acute hepatitis, 174
Acute liver failure (ALF), 41–43, 139–140
Acute-on-chronic liver failure (ACLF), 41–43
Acute rejection
  Banff grading scheme for, 386t
  descriptive terminology for, 387t
  prolonged, 387f
  vs. recurrent chronic hepatitis C, 394t
Acute (cellular) rejection, 383–385, 383f–384f
Acute viral hepatitis
  bile-duct epithelium, 95f
  bridging necrosis, 96f–97f
  chronic hepatitis, 103
  cirrhosis, 104
  delta hepatitis, 100–102, 101f
  differential diagnosis of, 103
  fatal outcome, 103
  hepatitis A, 97–99, 98f–99f
  hepatitis B, 99–100
  hepatitis C, 100, 101f
  hepatitis D, 100–102
  hepatitis E, 102, 102f
  hepatocellular carcinoma, 104
  hepatocellular damage, 90–91, 91f
  histological variants, 94–97
  inflammatory infiltrate, 92–94
  liver transplantation, need for, 103
  multilobular necrosis, 98f
  pathological features, 89–97
  periodic acid–Schiff, 93f
  Perls' stain-positive granules, 94f
  portal changes, 94
  portal tract, 95f
  reactivation, 100
  residual changes, 104f
  resolution, 103
  reticulin framework, 93f
  scarring, 103
  surviving hepatocytes, 90f
  types, 89, 90t
Acute yellow-fever hepatitis, 92f
Adenocarcinoma, 252–254
Adenofibroma, biliary, 214–215
Adenoid, 223
Adenoma
  bile-duct, 214–215, 214f
  hepatocellular, 206–210, 207f
    in children, 246–247
    diagnostic distinctions, 208–209, 208t
Adenoma (*Continued*)
    genomic changes, 206t
    inflammatory type, 209f
  subclassification, 207–208, 208t
Adenomatosis, 206–207, 209–210
Adenomatous hyperplasia, 219
Adenovirus, 343
Ageing, normal liver, 28–29
Ajmaline, 149–150
Alcohol aversion therapy, 141
Alcoholic fatty liver disease (AFLD), 114–118
  recurrence of, 394
Alcoholic foamy degeneration, 129
Alcoholic steatohepatitis (ASH), 121–126, 121f, 123f–124f
Allograft biopsy, 379–380
Alpha$_1$-antitrypsin deficiency, 174, 302–304, 302f–303f, 417f
Alpha-fetoprotein, 225–227
Amiodarone-induced phospholipidosis, 412f
Ammonia metabolism, 8
Amoebiasis, 355
Amoebic abscess, 356f
Amoxycillin, 149–150
Amyloid, 15
Amyloid amyloidosis, 267
Amyloidosis, 267, 349, 362–365, 363f–364f
Anabolic-androgenic steroid cholestasis, 147, 148f
Anabolic steroids, 212
Angiomyolipomas, 216–217, 217f, 227t
Angiosarcoma, 137t, 232–233, 232f, 252
Antibody-mediated rejection (AMR), 381–383, 382f
Anticonvulsant drugs, 139
Antimitochondrial antibodies (AMAs), 170

Note: Page numbers followed by "f" indicate figures, "t" indicate tables and "b" indicate boxes.

Apoptotic body, 37–38
Arsenic intoxication, acute, 141
Artefacts, 7–8, 7f
Arteries, 267–268
  relation to bile duct numbers, 194–195
Arteritis
  giant-cell, 267
  necrotising, 361
Ascariasis, 358
Augmentin, 149–150
Autoantibodies
  in autoimmune hepatitis, 170
  in non-alcoholic fatty liver disease, 116
Autoimmune cholangitis, 81–83, 82f
Autoimmune hepatitis (AIH), 76–77, 170–174, 171f–172f
  autoimmune disorders associated with, 175b
  definition, 170
  features of, 145b
  histological features, 170–172, 173b
  plasma cells, 196
  recurrence of, 393–394
Autoimmune sclerosing cholangitis, 293, 294f
Azathioprine, 390–391

B

Bacillary peliosis
  in AIDS, 349f
  hepatis, 273, 347–348
Bacterial cholangitis, 353
Bacterial infections, 349–353
Banff grading scheme, 386t
Behçet's disease, 212, 278
Benign lesions
  in adults, 206–217
  in children, 238
Benign recurrent intrahepatic cholestasis (BRIC), 311, 314f
β-catenin gene mutations, 207–208
Bile
  canaliculus, 418
  in hepatocellular carcinoma, 15
Bile-duct damage, 383, 398, 400
  causes of, 80–81, 81t
  differential diagnosis, 52, 52f
Bile-duct dysplasia, 72f
Bile-duct obstruction, 380
  with suppuration, 76–77
Bile ducts
  adenoma, 214–215, 214f
  carcinoma, 229–232, 230f
  cirrhosis, 194–195
  cystic, 215
  damage, 168, 172
  epithelium, 243, 244f
  loss, 194–195
    in biliary diseases, 174
    in childhood, 194–195
    drug-induced, 149–150, 194–195
  in neoplasia, 267
  normal liver, 21–22, 22f
  obstruction (*see* Biliary obstruction)
  paucity of, 295f–296f
  plate, differential diagnosis, 52, 52f
Bile ductular cholestasis
  differential diagnosis, 52, 52f
  in sepsis, 354f
Bile infarcts, 61, 62f
Bile-salt export pump (BSEP), 25, 25f, 225–227
Biliary adenofibroma, 214–215
Biliary cirrhosis, 64–66, 66f
Biliary cystadenoma, 215
Biliary disease, 174, 267
  biliary cirrhosis, 64–66, 66f
  cholangitis, 66–68
  cholestasis, 58–59
  chronic bile-duct obstruction, 64–66, 65f
  intrahepatic bile-duct loss, 83
  large bile-duct obstruction, 59–66, 65f
  primary biliary cholangitis, 73–83
  primary sclerosing cholangitis, 68–73, 69f
Biliary obstruction, 390
Biliary piecemeal necrosis, 156
Biliary tract disease, 10f
Biliary tree, 58, 59f
  infection of, 66–68
Biopsy of liver
  changes near metastatic tumour, 6f
  chemoembolic gels, 9f
  in chronic hepatitis, 156, 156b
  cirrhosis, 6f
Biopsy of liver (*Continued*)
  cytokeratin 7 immunostain, 10f
  differential diagnosis of pathological features, 34
  fixation artefact, 8f
  foreign material, 2, 3f
  fragmented biopsy, 34
  immunohistochemistry, 9f
  irregular contour, 34
  methods of obtaining, 136
  needles, 2–7
  normal contour, 34
  of normal liver, 29–31, 30f
  reasons for, 1–2, 1b
  routine microscopy, 34–35
  sample tissue schedules for, 14t
  specimen at bedside and in laboratory, 7–10
  specimen size, 187
  staining methods, 187b
  stains in evaluating, 15t–16t
  subcapsular necrosis, 5f
  techniques applicable to, 2–7, 2b
  transgastric, 5f
  transjugular *vs.* percutaneous needle biopsy, 4f
  transmission electron microscopy of, 408–411
  traumatic artefact, 7f
  type and adequacy of, 2–7
  variations in needle, 35f
  Yttrium 90 microspheres, 9f
Black cohosh, 173
Bland intrahepatic cholestasis, 44b
Blood vessels
  cirrhosis, 195
  neoplasms of, 206–207, 216–217
  tumours of, 205t, 216–217
Bone marrow transplantation, 400
Borderline nodule, 219, 220f
Breast carcinoma, 252–254
  immunohistochemical stains, 227t
Bridging necrosis, 94–95, 160–161
  acute viral hepatitis, 42f, 96f–97f
  hepatic, 42f
  viral hepatitis, 96f–97f
Brucellosis, 349
Budd–Chiari syndrome, 211, 276, 282
Busulfan, 282
Byler disease, 309
Bypass channels, 280

C

Calcifying nested stromal–epithelial tumour, 240, 242f
Canalicular cholestasis, 44, 44b, 353
Candidiasis, 352
Carbon tetrachloride, 137
Carcinoma
  bile ducts, 229–232, 230f
  breast, 227t, 252–254
  caval obstruction and, 278
  in cirrhosis, 187
  colon, 252–254
  colorectal, 227t
  fibrolamellar, 206t, 221–223, 228–229, 229f, 251f
  hepatocellular, 187, 191–193, 191f, 202, 220–228, 395–396
    acute viral hepatitis, 104
    adenoid pattern, 223, 223f
    in adults, 220–228
    in children, 240–242, 247–250, 248f–250f
    cytological grading, 225f
    diagnosis of, 225–227
    fibrolamellar type, 229f
    fine-needle aspiration biopsy features of, 250b
    genomic changes, 206t
    immunohistochemical stains, 227t
    in non-alcoholic steatohepatitis, 271
    in non-cirrhotic liver, 212
    precursors of, 217–219
    reticulin is scanty, 222f
    scirrhous, 222f
    steato-hepatitic variant of, 224f
    trabecular–sinusoidal structure, 221f
  hepatoid, 234–235
  lung, 227t
  metastatic, 6f
  sclerosing, 223
Cardiac sclerosis, 276–277, 279f
Cardiolipin antibody, 273
Caroli's disease, 72–73, 229–230, 298
Caseating granuloma, 339
Cat-scratch disease, 350
Caval obstruction, 278
Cell sap, 418
Central perivenulitis/venulitis, 383–385
Central venulitis, 398
Ceroid-laden Kupffer cells, 53, 53f
Ceroid pigment, 276–277
  definition, 141
Checkpoint inhibitors, 138
Chemoembolic gels, liver biopsy, 9f
Childhood
  benign lesions in, 238
  focal nodular hyperplasia in, 206–209, 246
  hepatocellular adenomas in, 246–247
  liver tumours in, 209–210
  malignant lesions in, 239–242
  neoplasms in, 238–242
  nodular regenerative hyperplasia in, 238
  nodules in, 238–242
  normal liver appearances in, 27
  vanishing bile duct syndromes in, 194–195
Chlorpromazine, 149–150
Chlorpropamide, 149–150
Cholangiocarcinoma, 229–232, 230f, 250
  genomic changes, 206t
  immunohistochemical stains, 227t
  in primary sclerosing cholangitis, 71f
Cholangiolocellular carcinoma, 230–232, 231f
Cholangiopathy, 347
Cholangitis. *see also* Primary biliary cholangitis
  acute, 67f
  autoimmune, 81–83, 82f
  bacterial, 353
  infection of biliary tree, 66–68
  non-bacterial, 353
  sclerosing, 267
Cholate stasis, 64–65
Choledochal cysts, 229–230, 293
Cholestasis, 58–59, 137t, 236, 322–323, 390–391, 399–400
  acute, 44
  anabolic-androgenic steroid, 147, 148f
  benign recurrent intrahepatic, 311, 314f
  bile ductular, 52, 52f
Cholestasis (*Continued*)
  bile thrombi, 60f
  biliary disease, 58–59
  bland intrahepatic, 44b
  canalicular, 44, 44b, 353
  chronic, 50–51, 64–65, 65f
  copper in, 322–323
  differential diagnosis, 44–51, 45f, 47f, 147
  drug-induced, 147–150
  ductular, 353
  functional, 379
  idiosyncratic drug-induced, 147–150, 149f
  intrahepatic, 58–59, 58b, 368
  pregnancy, 368
  in septicaemia, 60f
  steroid-induced, 147
  swollen hepatocytes, 61f
Cholesterol ester storage disease, 305
Chondroma, 216–217
Chronic bile-duct obstruction, 64–66, 65f
Chronic biliary diseases, 174
Chronic cholestasis, 50–51, 64–65, 65f
Chronic drug-induced hepatitis, 143–145, 144f
Chronic graft-*versus*-host disease, 400
Chronic hepatitis, 72–73, 80–81, 155–181, 349, 365, 379
  acute viral hepatitis, 103
  in AIDS, 348
  autoimmune hepatitis, 170–174
  causes, 155, 155b
  cirrhosis in, 193
  classification and nomenclature, 155
  definition, 155
  differential diagnosis, 174
  ductular reaction in, 160f
  grading, 178f
  grading and staging, 174–181, 175b–176b, 176t–177t, 178f–180f
  hepatitis B, 163–167
  hepatitis C, 167–170
  hepatitis D, 167
  hepatitis E, 170
  histologic features of, 156–163, 168b
  hyaline droplets, 163, 164f

Chronic hepatitis (*Continued)*
individual causes, 163–174
iron overload, 333–335
Ishak scoring system, 175, 177t
liver biopsy in, 156, 156b
with lobular activity, 161f
METAVIR staging system, 175, 176b
oncocyte formation, 163f
parenchymal changes, 156–163
portal changes, 156, 157f
postinfantile giant-cell transformation, 162f
with rosette formation, 162f
scoring system for, 175b, 178–181
steatosis, 169
in Wilson's disease, 174
Chronic inflammatory bowel disease, 359–360
Chronic lymphocytic leukaemia, 237
Chronic (ductopenic) rejection, 385–389, 388f
Ciclosporin, 390–391
Ciprofloxacin, 149–150
Cirrhosis, 130, 146–147, 146f–147f, 187–197, 200–202, 243–246, 245f, 365
abnormal deposits, 196–197
abnormal vascular relationships, 188–189, 190f
active, 201, 202f
activity, 201
acute viral hepatitis, 104
after hepatitis, 321
anatomical types, 197
assessment of cause, 194–197, 194b
biliary (*see* Biliary cirrhosis)
carcinoma in, 187
cause, 194b
in childhood, 311–313 (*see also* Childhood)
in chronic hepatitis, 193
complications, 202
congestive, 274
developing, 200–201, 200f
diagnosis, 187–193, 188b
different cell populations, 191, 192f
differential diagnosis, 193
distorted reticulin pattern, 188–189, 190f
Cirrhosis (*Continued)*
evidence of viral infection, 196
fragmentation of, 187–188, 188f
hepatocellular changes, 191–193, 191f
inactive, 201, 201f
incomplete, 200–201
incomplete septal, 197, 199f–200f
liver biopsy in, 6f, 187–193, 187b
macronodular, 199f
micronodular, 198f
in non-alcoholic steatohepatitis, 194
primary biliary and chronic hepatitis, 212
reversed lobulation, 194, 195f
sampling error, 2–3
with siderosis, 334f–335f
stage of development, 200–201
venous occlusion in, 197
Wilson's disease, 321, 322f
Classic HFE-related hereditary haemochromatosis, 327–330, 328t
Clavulanic acid, 149–150
Clonorchis sinensis, 357
Coagulative necrosis, 39, 277f
Cocaine, 142
Coeliac disease, 358–359
Coexisting liver disease of other aetiology, 326
Collagen, 218f, 273, 280
liver in, 361–362
measurement, 174
stains for, 14
Collapse/condensation of reticulin, 14, 267–268
Colon carcinoma, 252–254
Colorectal carcinoma, 227t
Combined hepatocellular–cholangiocarcinoma, 223
Common variable immunodeficiency, 341–342
Confluent necrosis, 40–43, 94–95, 160–161
Congenital extrahepatic portosystemic shunts. *see* Abernethy malformation
Congenital hepatic fibrosis, 193, 297, 297f
Congestive cardiac failure, 268, 274, 276
Congestive hepatopathy, 276–278, 279f, 280t
ductular reaction associated with, 278f
fibrosis scoring system for, 280t
histologic features, 277b
in sickle-cell disease, 276–278, 277b, 278f–279f, 280t
Contraceptive steroids, 147
Copper, 15
in cholestasis, 322–323
in cirrhosis, 193, 196–197
stain for, 323, 325f
in Wilson's disease, 322–323, 325f
Copper-associated protein, 51, 193, 196–197
in cholestasis, 322–323
stains for, 15
Co-trimoxazole (trimethoprim-sulphamethoxazole), 140–141
Councilman body, 37–38
CRST syndrome, 361
Cryptogenic cirrhosis, 127f
Crystals and spicules, 168
Cyanamide toxicity, 163–166
Cylindrical confronting cisternae, 421
Cystadenocarcinomas, 232
Cystadenoma, biliary, 215
Cystic fibrosis (CF), 299–300, 300f
Cysts, 273
bile ducts, 215
blood, 273
choledochal, 229–230
liver, 299f
Cytokeratin 7 immunostain, 10f
Cytokeratins, Mallory–Denk bodies, 321
Cyto-megalovirus hepatitis, 390f
Cytomegalovirus infection (CMV), 343–344, 345f
Cytoplasmic inclusion bodies, 141
Cytosol, 418

D

Damage-associated molecular pattern (DAMP), 141
Delta hepatitis, 100–102, 101f
Dengue fever, 343
De novo autoimmune hepatitis, 394–395, 395f
Diabetes mellitus, 118, 119f

Diabetic hepatosclerosis, 118, 120f
Diagnostic tests, 328
Diclofenac, 138
Didanosine, 139–140, 140f
Differential diagnosis of pathological features
  abnormal macrophage pigment, 56
  bile-duct damage, 52
  bile-duct plate, 52
  bile ductular cholestasis, 52
  ceroid-laden Kupffer cells, 53
  cholestasis, 44–51
  coagulative necrosis *vs.* apoptosis, 37f
  confluent necrosis, 40–43
  ductular reaction, 46–51
  erythrophagocytosis, 53
  extramedullary haemopoiesis, 54
  hepatocellular damage, 37–44
  inflammatory cell infiltration, 54–56
  interface hepatitis, 43–44
  naked-eye examination, 34
  patterns of injury, 35–51
  perisinusoidal fibrosis, 54
  portal tract size variations, 36f
  routine microscopy, 34–35
  sinusoidal congestion, 53
  structural changes, collapse and fibrosis, 35–37
Direct hepatotoxins, 137
Disseminated intravascular coagulation, 274, 275f
Distribution of stainable iron, 326
Down syndrome, 360
  with transient abnormal myelopoiesis, 362f
Drop-out, liver-cell, 172–173
Drug-induced granuloma formation, 150f
Drug-induced liver injury
  hepatitic type, 142, 142f–144f, 143b
  hepatocanalicular type, 149f
Drug injury, 76–77, 174
Drug-related hepatotoxicity, 346
Drugs and toxins, 136–151, 342
  acute drug-induced hepatitis, 142–143
  adaptation, 139, 139f
  chronic drug-induced hepatitis, 143–145
  classification and mechanisms of, 137–138
  commonly implicated and newer agents, 138
  fibrosis and cirrhosis, 146–147
  granulomas, 150
  hepatocellular necrosis, 141–142
  idiosyncratic drug-induced cholestasis, 147–150
  liver lesions due to, 137t
  morphological categories, 138–151
  non-hepatitic liver-cell damage, 139–142
  steatohepatitis, 145–146
Drug toxicity, 380, 390–391
Dubin–Johnson syndrome, 207, 308, 308f, 411f
Ductal plate malformation, 193
Ductopenia, 194–195
  in biliary diseases, 172
  in childhood, 194–195
  drug-induced, 143–145, 194–195
  idiopathic adulthood, 83, 293–294
Ductular cholestasis, 353
Ductular reaction, 46–51
  in chronic hepatitis, 160f
  with congestive hepatopathy, 276–277, 278f
  with cytokeratin 7 immunohistochemistry, 50f
  in different diseases, 48f–49f
  in focal nodular hyperplasia, 211, 212f
  large bile-duct obstruction, 63f
Dysmetabolic iron overload syndrome (DIOS), 117, 119f, 326, 335
Dysplasia, bile-duct, 72f
Dysplasia, liver-cell
  definition, 218
  large cell, 191–193, 192f
  small cell, 191–193, 193f
Dysplastic foci, 202, 218–219
Dysplastic nodules, 202, 219, 220f

E

'Ecstasy' (3,4-methylene-dioxymethamphetamine), 142
Elastic fibres, 14–15
Electron microscopy
  gene array, 424–426
  gene sequencing, 424–426
  immunoelectron microscopy, 422
  immunohistochemistry, 423–424
  of liver biopsies, 408–411
  molecular analysis, 424–426
  scanning electron microscopy, 423
  ultrastructural changes, 411–422
  in Wilson's disease, 321
Embryonal rhabdomyosarcoma, 240
Endoplasmic reticulum, 415
  hypertrophy, 163–166
Endothelial cell, 420–421
Endotheliitis, 383, 398
  in acute rejection, 385f
Entamoeba histolytica, 355
Eosinophilic gastroenteritis, 358–359
Eosinophilic granules, 273
Eosinophils, 55, 398–399
Epithelial hepatoblastoma, 239–240
Epithelioid haemangioendothelioma, 233, 233f–234f, 282
  immunohistochemical stains, 227t
Epithelioid-cell granulomas, 168
Epstein–Barr virus, 217, 389
Erythromycin, 149–150
Erythrophagocytosis, 53, 53f
Erythropoietic protoporphyria, 366f
Essential mixed cryoglobulinaemia, 361
Exertional heatstroke, 352
Extracellular biliary atresia (EHBA), 289–293
Extracellular vesicles, 422
Extra-hepatic biliary atresia, 291f
  with bile-duct plate-like structures, 292f
  histological predictors of, 291b
Extrahepatic malignancy, 234–237
Extramedullary haemopoiesis, 54, 54f, 360, 361f

F

Factor VIII-related antigen, 221–223
Fanconi's anaemia, 209–210
Fascioliasis, 76–77, 359f
Fat-storing cells. *see* Stellate cells
Fatty liver, 379
  Wilson's disease, 321, 322f
Feathery degeneration, 38, 59–61
Felty's syndrome, 361
Ferroportin-related iron overload, 331
Ferrous sulphate toxicity, 137
Fetal alcohol syndrome, 130
Fialuridine, 139–140
Fibrin-ring granulomas, 339, 342, 349
Fibrolamellar carcinoma, 221–223, 228–229, 229f, 251f
  genomic changes, 206t
Fibropolycystic diseases, 296–299
Fibrosing cholestatic hepatitis, 391, 392f
Fibrosis, 129–130, 137t, 146–147, 146f–147f, 276–278, 280
  absence of, 213f
  in chronic hepatitis, 159f
  chronic hepatitis with, 159f
  congenital hepatic, 193, 297, 297f
  drug-induced, 146
  pericellular, 124f
  periductal, 195
  perisinusoidal, 54, 54f, 146
  scoring system for, 280t
  of terminal hepatic venules, 390–391
Fine-needle aspiration biopsies (FNABs), 206–207, 250b
Fixation artefact, 8f
FK 506, 390–391
Flavivirus infection, 343
Flucloxacillin, 149–150
Focal fatty change, 111, 111f
Focal glycogenosis, 306, 306f
Focal necrosis, 38–39, 160–161
  vs. spotty necrosis, 41f
Focal nodular hyperplasia (FNH), 210–211
  in children, 206–209, 246
  diagnostic distinctions, 208–209, 208t
  identification of, 238
  needle biopsies of, 212f
Focal nodular hyperplasia (FNH) (*Continued*)
  small bile-duct-like structures, 211f
  telangiectatic, 209f, 210
Foci
  dysplastic, 202
  iron-free, 218–219
Follicles, 55–56, 55f
Foreign material, liver biopsy, 2, 3f
Fragmentation, of cirrhosis, 187–188, 188f
Frozen section of potential donor livers, 379
Functional cholestasis, 379
Functional heterogeneity, of liver, 8, 9f
Fungal infections, 349–353

G

Galactosaemia, 305–306
Gastrointestinal disorders, 358–360
Gaucher's disease, 304, 304f, 408–409, 410f
Gemtuzumab-ozogamicin, 282
Gene array, 424–426
Gene sequencing, 424–426
Giant-cell arteritis, 267
Giant-cell hepatitis, 349
Gilbert's syndrome, 308
Glue sniffing, 142
Glutamine synthetase, 8, 9f
Glycogen, 15, 419
Glycogenic hepatopathy, 118, 120f, 301–302
Glycogenosis, 301–302, 301f
Glycogen storage diseases, 301–302, 301f, 421f
Glycoproteins, 15
Glycosyl ceramide lipidosis, 304, 304f
Glycyrrhizin, 149–150
Gold compounds, 139
Golgi apparatus, 418
Grade of siderosis, 326
Grading
  of acute rejection, 385
  of chronic hepatitis, 174–181, 178f
  of histological activity in hepatitis, 174–181
Graft rejection, 380–389
  acute (cellular) rejection, 383–385, 383f
  antibody-mediated rejection, 381–383, 382f
  chronic (ductopenic) rejection, 385–389
  infection, 389–390
  sinusoidal foam cells, 389f
Graft-*versus*-host disease (GVHD), 76–77, 400
  bile-duct epithelium, 401f
  haemolysis, 381–382
Gram-negative bacilli, 389–390
Granules, in sinusoidal endothelial cells, 273
Granulomas, 150, 150f, 339–343, 352
  in adenomas, 207
  with central stellate microabscesses, 350
  in childhood, 341–342
  in giant-cell arteritis, 267
  in hepatitis, 168
  histological features of, 340t
  in inflammatory pseudotumour, 217, 218f
  mycobacterial and fungal infections producing, 346–347
  in systemic lupus erythematosus, 267
Ground-glass hepatocytes, 38t, 39f, 163–166
  chronic hepatitis B, 163–166, 164f
  definition, 166
Ground-glass-like hepatocellular inclusions, 400

H

Haemangioendothelial sarcoma. *see* Angiosarcoma
Haemangioendothelioma
  epithelioid, 233, 233f–234f, 282
  immunohistochemical stains, 227t
  infantile, 239, 239f
Haemangioma, 215–216, 215f, 247, 248f
Haematological disorders, 360
Haematoma in HELLP syndrome, 369

Haemochromatosis, 220–221
Haemolysis, 322–323
Haemophilia, 360
Hairy cell leukaemia, 237
Haloperidol, 149–150
Hamartoma
  biliary microhamartoma, 214–215
  mesenchymal, 238–239, 238f
Hantavirus, 343
Heart failure, 267–268
Heatstroke, 267–268, 269f
HELLP syndrome, 366–367
Hepatic arteries, 267–268
Hepatic failure, late onset, 322–323
Hepatic iron concentration (HIC), 327
Hepatic sinusoids, 273–276
  normal liver, 23
Hepatic small vessel neoplasms, 215–216
Hepatic stellate cell (HSC), 421–422
Hepatic tumours, 227t
Hepatic veins, obstruction of, 278
Hepatic venous outflow obstruction. *see* Venous outflow obstruction
Hepatitis, 137t
  acute drug-induced, 142–143, 142f–143f, 143b
  autoimmune, 170–174, 171f–172f
    definition, 170
    histological features, 170–172, 173b
    plasma cells, 196
  chronic, 155–181
    causes, 155, 155b
    classification and nomenclature, 155
    definition, 155
    differential diagnosis, 174
    grading and staging, 174–181, 175b–176b, 176t–177t, 178f–180f
    histologic features of, 156–163, 168b
    hyaline droplets, 163, 164f
    individual causes, 163–174
    liver biopsy, uses in, 3–4, 156, 156b
    steatosis, 169
    in Wilson's disease, 174
Hepatitis (*Continued*)
  chronic drug-induced, 143–145, 144f
  fatty liver (*see* Steatohepatitis)
  granulomas in, 168
  interface in chronic hepatitis, 156–160, 158f–159f
  ischaemic, 268
  lupoid (*see* Hepatitis, autoimmune)
  peliosis, 273, 274f
  steatosis in, 169
  viral
    bile-duct epithelium, 95f
    bridging necrosis, 96f–97f
    chronic hepatitis, 103
    cirrhosis, 104
    delta hepatitis, 100–102, 101f
    differential diagnosis of, 103
    fatal outcome, 103
    hepatitis A, 97–99, 98f–99f
    hepatitis B, 99–100
    hepatitis C, 100, 101f
    hepatitis D, 100–102
    hepatitis E, 102, 102f
    hepatocellular carcinoma, 104
    hepatocellular damage, 90–91, 91f
    histological variants, 94–97
    inflammatory infiltrate, 92–94
    liver transplantation, need for, 103
    multilobular necrosis, 98f
    pathological features, 89–97
    periodic acid–Schiff, 93f
    Perls' stain-positive granules, 94f
    portal changes, 94
    portal tract, 95f
    reactivation, 100
    residual changes, 104f
    resolution, 103
    reticulin framework, 93f
    scarring, 103
    seroconversion, 166
    surface antigen (HBsAg), 163–166, 165f
    surviving hepatocytes, 90f
    type B, 157f, 163–167, 164f–166f, 196f
    types, 89, 90t
Hepatitis A, 97–99, 98f–100f
Hepatitis B
  chronic, 163–167, 164f, 348
  mild, 157f
  recurrent, 391
  viral hepatitis, 99–100
Hepatitis C
  acute, 267–268
  chronic, 167–170, 168f–169f, 168b, 342, 348
  granulomas in, 168
  immunohistochemistry, 169
  viral hepatitis, 100, 101f
  virus infection, cirrhosis following, 197f
Hepatitis D
  chronic, 167
  viral, 100–102
Hepatitis E, 170
  chronic, 170
  viral hepatitis, 102, 102f
Hepatobiliary mucinous cystic neoplasm, 215
Hepatoblastoma, 239–240, 240t
  cell cluster, 251f
  embryonal epithelial type, 241f
  epithelial type of, 250
  fetal epithelial type, 241f
  genomic changes, 206t
  immunohistochemical stains, 227t
  mesenchymal type, 250
Hepatocellular adenomas (HCAs), 206–210, 207f
  in children, 246–247
  diagnostic distinctions, 208–209, 208t
  genomic changes, 206t
  inflammatory type, 209f
Hepatocellular ballooning, 38, 380
Hepatocellular carcinoma (HCC). *see* Carcinoma, hepatocellular
Hepatocellular damage, 37–44
  acute viral hepatitis, 90–91, 91f
Hepatocellular necrosis, 141–142, 141f–142f
Hepatocellular pigments, 25–27, 26t, 27f
Hepatocytes, 412–420
  adaptation, 139, 139f
  ballooning, 40f, 116f
  drop-out, 172–173
  dysplasia, 218
  eosinophilic inclusions, 161

Hepatocytes (*Continued)*
feathery degeneration, 147
lamellar inclusions, 140–141
large-cell change, 191–193
normal liver, 24–25, 25f
normal/reactive, 242–243, 243f–244f
nuclei (*see* Nuclei, hepatocellular)
oncocytic change, 161
premalignant changes, 217–218
small-cell change, 218
swelling, 38, 40f
Hepatoid carcinomas, 234–235
Hepatoma. *see* Carcinoma, hepatocellular
Hepatoportal sclerosis, 271
Hepatosclerosis. *see* Diabetic hepatosclerosis
Hepatotoxicity
classification, 137–138
direct, 137
idiosyncratic, 137, 137t
indirect, 137
intrinsic, 137–138, 137t
Herbal medicines, 136
Hereditary fructose intolerance, 306
Hereditary haemochromatosis, 329f–330f
Hereditary haemorrhagic telangiectasia, 267
Heroin, 273, 282
Herpes simplex virus (HSV) hepatitis, 343, 344f
HFE-related hereditary haemochromatosis, 327–330, 328t
Highly active antiretroviral therapy (HAART), 344–346
High mobility group box-1 (HMGB1), 141
Histological grading of hepatocellular iron, 327
Histological variants, acute viral hepatitis, 94–97
Histology Activity Index (HAI), 175
Histoplasma, 355
Histoplasmosis, 352
HMB-45, immunostaining, 216–217
Hodgkin's disease, 83, 235–236, 236f
Hyaline arteriolosclerosis, 118, 267
Hyaline droplets, 163, 164f
Hyalin Mallory's. *see* Mallory bodies
Hydrochlorofluorocarbons, 142
Hyperbilirubinaemias, 308
Hypertrophied perisinusoidal stellate cells, 349
Hypervitaminosis A, 146, 146f
Hypoperfusion, hypotension, 202

I

Idiopathic adulthood ductopenia, 83, 293–294
Idiopathic portal hypertension, 271
Idiopathic posttransplantation hepatitis (IPTH), 397, 398f
Idiosyncratic drug-induced cholestasis, 147–150, 149f
Idiosyncratic hepatotoxicity, 137, 137t
Idiosyncratic (unpredictable) hepatotoxins, 137, 137t
Image analysis, 181
Immune checkpoint inhibitors, 138
Immune-complex diseases
liver in, 361–362
Immune reconstitution, in AIDS, 348
Immunoelectron microscopy, 422
Immunoglobulin G4 (IgG4)-associated cholangitis (IAC), 72–73, 73f
Immunohistochemical staining, 15
Immunohistochemical (IHC) stains
bile canalicular structures, 228f
in hepatic tumours evaluation, 227t
primary malignant liver tumours, 226f
Immunohistochemistry, 8, 9f, 423–424
identification of pathogens, 163–166
of liver lobule, 9f
Immunosuppression withdrawal, 391
Inclusion bodies, in hepatocytes, 141
Incomplete septal cirrhosis, 197, 199f–200f
Indian childhood cirrhosis, 313, 314f
Indirect hepatotoxins, 137
Indometacin, 142f
Industrial chemicals, 136
Infantile haemangioendothelioma, 239, 239f
Infarcts, 267, 269f
nodules, 202
Zahn's, 270
Infections and infestations, 346–347
Infectious mononucleosis, 344, 345f
Inferior vena cava, obstruction of, 278
Inflammation, 353
Inflammatory cell infiltration, 54–56
Inflammatory infiltrate, 92–94
Inflammatory myofibroblastic tumour (IMT), 217
Inflammatory pseudotumour, 217, 218f
Inherited Cholestatic syndromes, 308–311
Inherited metabolic disorders, 299–307
Inset, 382–383
Insulin resistance-iron overload. *see* Dysmetabolic iron overload syndrome (DIOS)
Intercurrent liver disease during pregnancy, 366
Interface hepatitis, 43–44, 156–160, 158f–159f
Intrahepatic bile ducts
in childhood, 293–296
non-syndromatic paucity of, 83
Intrahepatic cholestasis
causes of, 58–59, 58b
of pregnancy, 368
Intrahepatic cholestasis of pregnancy (ICP), 366
Intrinsic hepatotoxicity, 137–138, 137t
Intrinsic (predictable) hepatotoxins, 137, 137t
Iron, 15, 15t–16t, 321–323, 326–335
classification, 275–276
in endothelial cells, 169
in steatohepatitis, 218
Iron-free foci, 218–219
Iron overload
in haematological disorders, 333
numerical assessment, 327
primary disorders, 327–331

Iron overload (*Continued*)
secondary disorders, 331–335, 331f
siderosis, 326
Irradiation, 282
Ischaemic hepatitis, 268
Ishak scoring system, chronic hepatitis, 177t
Isolated central perivenulitis, 383–385, 396, 397f
Isolated ductular hyperplasia, 64
Isolated sinusoidal megakaryocyte, 54, 54f
Isoniazid, 137t
Ito cells. *see* Stellate cells

J

Jaundice
cholestatic of pregnancy, 147

K

Kala-azar, 355, 356f
Keratin 18, 141
Keratins, gene mutations, 194
Klatskin tumour, 229–230
Knodell Histology Activity Index (HAI), 175
Kupffer cell, 420
Kupffer cells
in biliary obstruction, 275–276
in drug injury, 141
hyaline droplets in, 163, 164f
Kupffer-cell siderosis, 360, 381–382

L

Laboratory techniques
choice of stains, 14–15
processing of specimen, 13
Langerhans cell histiocytosis (LCH), 237, 293
Langerhans cell histiocytosis i, 295f
Large bile-duct obstruction, 59–66
with bile infarct, 62f
ductular reaction, 63f
phagocytic reaction, 65f
portal tract, 62f
prominent ductular reaction, 63f
Large-cell liver-cell dysplasia, 218
Large granular lymphocyte, 422
Larval diseases, 358
Late liver-allograft dysfunction, 396–397
Leishmania donovani, 355
Leprosy, 351
Leptospirosis, 352
Leukaemias, 235–237
Light-chain deposition, 362–365
Lipocytes. *see* Stellate cells
Lipogranulomas, 112, 112f, 168, 339
Lipoma (pseudolipoma), 216–217
Lipopeliosis, 112, 113f, 380, 381f
Liver
extrahepatic malignancy and, 234–237
normal/reactive, 242–243
Liver abscess, 353
Liver biopsy. *see* Biopsy of liver
Liver cells. *see* Hepatocytes
Liver-cell adenoma, 238
Liver-cell dysplasia (LCD), 218, 243–246, 246f
Liver disease
alcoholic fatty (*see* Alcoholic fatty liver disease (AFLD))
coexisting, 326
macrovesicular fatty, 114–118, 115f
non-alcoholic fatty (*see* Non-alcoholic fatty liver disease (NAFLD))
pre-existing, 366
of varied aetiology, 333–335
Liver flukes, 357–358
Liver lesions
in alcoholic, 129b
due to drugs and toxins, 137t
LiverTox, 136
Liver transplantation
allograft biopsy, 379–380
assessment of donor liver, 378–379
bone marrow transplantation, 400
differential diagnosis, 397–400, 399t
graft dysfunction, 389–397
graft rejection, 380–389
liver-cell ballooning after, 381f
pathological lesions after, 380f
renal transplantation, 400
Liver tumours
classification of, 205, 205t
genomic changes associated with, 205, 206t
Lobular activity, chronic hepatitis with, 161f
Lobular hepatitis, 160–161, 161f
Lobular lesion, 160–163
Loss of interlobular bile ducts, 51
Lung carcinoma, immunohistochemical stains, 227t
Lyme disease, 352
Lymphangioma, 215–216
Lymphoepithelioma-like cholangiocarcinoma, 230–232
Lymphoid aggregates, 55–56, 55f
and follicles, 169–170, 197f
in hepatitis C, 196, 197f
Lymphomas, 174, 235–236, 240, 252, 252f
in AIDS, 348
mucosa-associated lymphoid tissue, 236–237
non-Hodgkin's, 236–237, 237f
peripheral γ-δ/αβ T-cell lymphomas, 236–237
primary hepatic, 236–237
Lymphoproliferative disease, 380
Lysosomes, 417

M

Macroglobulinaemia, 237
Macrophage activation syndrome, 360
Macroregenerative nodules (MRNs), 202, 219, 219f
Macrotrabecular pattern, 240
Macrovesicular fatty liver disease, 114–118, 115f
Macrovesicular steatosis, 108–112
causes of, 109b
fat vacuoles, 108f
with focal inflammation, 115f
swollen hepatocytes, 109f
Malacoplakia, 361–362
Malaria, 353–355, 354f
Malignant lesions
in adults, 217–233
in children, 239–242
Malignant melanoma, 252–254

Mallory bodies
in Indian childhood cirrhosis, 196–197
possible premalignant significance, 218–219
in steatohepatitis, 195–196
Mallory–Denk bodies, 121–122, 122f, 224–225, 419f
in adenomas, 207
drug-induced, 168
in hepatitis C, 168
Wilson's disease, 321, 323f
Massive hepatic necrosis, 41–43
Mass lesions, 379
Mauriac syndrome, 301–302
with glycogenic hepatopathy, 120f
MDMA (Ecstasy), 142
Megasinusoids, 271
Menghini needle, 2
Mesenchymal hamartoma, 238–239, 238f
genomic changes, 206t
Mesenchymal stroma, in biliary cystadenoma, 215
Mesenchymal tumours, 216–217
Mesothelium, 243, 245f
Meta-static colonic adenocarcinoma, 253f
Meta-static neuroendocrine carcinoma, 253f
Metastatic tumours, 234–235, 235f, 252–254
Meta-static uveal melanoma, 254f
METAVIR staging system, 175, 176t, 176b
Methotrexate, 146–147, 147f
Methyldopa, 143–145, 144f
3,4-Methylenedioxymethyl-amphetamine (Ecstasy), 142
Microabscesses, 352
Microabscess-like foci, 389
Microhamartoma, 298, 298f
Microscopy
electron (*see* Electron microscopy)
in Wilson's disease, 321
Microvesicular steatosis, 109f, 112–114, 137t, 139–140, 140f
causes of, 114b
didanosine-induced, 140f
drug-related, 139–140
Microvesicular steatosis (*Continued*)
non-hepatitic liver-cell damage, 139, 140f
Mini-microabscesses, 389
Minocycline, 173
Mitochondria, 413–414
Mitochondriopathy, 416f
Mixed connective tissue disease, 361–362
Mixed epithelial–mesenchymal hepatoblastoma, 239–240
Molecular analysis, 424–426
Mucosa-associated lymphoid tissue (MALT) lymphoma, 236–237
Mucoviscidosis. *see* Cystic fibrosis
Multiacinar necrosis, 96–97
Multilobular necrosis, 96–97
acute viral hepatitis, 98f
Multilobular (multiacinar) necrosis, 41–43
Multiple myeloma, 237
Mycobacterium avium–intracellulare, 346–347, 348f
Mycobacterium tuberculosis, 346–347
Myelolipoma, 216–217
Myeloproliferative disorders, 270, 278

N

NAFLD. *see* Non-alcoholic fatty liver disease
Naked-eye examination, 34
NASH. *see* Non-alcoholic steatohepatitis
Near-normal liver, 34
Necrosis, 206–207, 267, 271, 275–276
bridging, 96f–97f, 160–161
bridging hepatic, 42f
coagulative, 277f
confluent, 160–161
drug- and toxin-related, 142
drug-induced, 147–148
focal, 160–161, 201
focal *vs.* spotty, 41f
hepatocellular, 141–142, 141f
panlobular/multilobular, 41–43
panlobular/panacinar, 160
periportal, 137, 274, 276–277
piecemeal (*see* Hepatitis; interface)
spotty, 160–161
submassive, 43f
subscapular, 5f
Needle biopsy
nodular regenerative hyperplasia, 213f
transjugular *vs.* percutaneous, 4f
Neonatal haemochromatosis, 332, 332f
Neonatal hepatitis, 288–289, 289f–290f
Neonatal liver biopsy, 287–288
Neoplasms
adenoma, bile-duct, 214–215, 214f
in adults, 206–233
biliary adenofibroma, 214–215
of blood vessels, 206–207, 216–217
in children, 238–242
cholangiocarcinoma (*see* Bile ducts, carcinoma)
cytopathological diagnosis, 242–254
epithelioid haemangioendothelioma, 282
extrahepatic, liver changes in, 234–237
hepatocellular carcinoma (*see* Carcinoma, hepatocellular)
lymphangioma, 215–216
malignant, 217–233, 239–242
mesenchymal and neural, 216–217
neuroendocrine, 212
neurofibroma, 216–217
Neoplastic disease, 395–396
Neural tumours, 216–217
Neuroendocrine
carcinomas, 252–254
immunohistochemical stains, 227t
Neurofibroma, 216–217
Neutrophils, 54–55, 267–268, 275f, 321, 398–399
in steatohepatitis, 321
Niemann–Pick disease, 305, 305f, 410f
Nimesulide, 138
Nitrofurantoin, 143–145
Nodular infarction, 202
Nodular lesions
classification of, 205, 205t
Nodular regenerative hyperplasia (NRH), 151, 193, 211–214, 272, 349, 361

Nodular regenerative hyperplasia (NRH) (*Continued*)
absence of fibrosis, 213f
anticardiolipin antibody, 212
cardiolipin antibody, 273
with centrilobular sinusoidal dilatation and cardiac sclerosis, 279f
in children, 238
in coeliac disease, 212
needle biopsy, 213f
wedge liver biopsy, 211–212, 213f
Nodular transformation, 214
Nodules, 187, 189f
in adults, 206–233
borderline, 219, 220f
in children, 238–242
dysplastic, 219, 220f
focal nodular hyperplasia, 206–207
infarction, 267
partial nodular transformation, 214
Non-alcoholic fatty liver disease (NAFLD), 114–118, 117f
recurrence of, 394
Non-alcoholic steatohepatitis (NASH), 125–126, 126f–127f, 128t, 145–146
Non-bacterial cholangitis, 353
Non-caseating granuloma, 339
Non-cirrhotic portal fibrosis/hypertension, 271, 272f
Non-hepatitic liver-cell damage, 139–142
Non-HFE haemochromatosis, 331
Non-Hodgkin's lymphoma, 236–237, 237f
Non-specific reactive changes, 365
Non-steroidal anti-inflammatory drugs (NSAIDs), 136
Non-syndromatic intrahepatic bile ducts, 293–294
Normal liver
ageing, 28–29
bile ducts, 21–22, 22f
biopsy, 30f
biopsy of, 29–31
CD34 vascular endothelial staining, 23, 23f
checklist of pertinent negatives, 31b
copper in, 321
elderly person, 29f
Normal liver (*Continued*)
functional units and nomenclature, 20
hepatic sinusoids, 23
hepatocellular pigments, 25–27, 26t, 27f
hepatocytes, 24–25, 25f
lipofuscin pigment, 27f
neonate, 28f
normal appearances in childhood, 27
operative wedge biopsy, 31f
portal tracts, 20–21, 21f
space of Disse, 23–24
structures and components, 20–27
19 weeks' gestation, 28f
Notch1 signalling, 214
Nuclei, hepatocellular
glycogen vacuolation, 321
multiple, 216–217
in large-cell change, 218
sanded, 166, 166f
Nucleus, 412

O

Obliterative cavopathy, 278
Obliterative portal venopathy, 271
Oestrogens, toxic effects, 145–146
Oncocytes, 161, 163f
Operational tolerance, 391
Operative wedge biopsy, 31f
Opisthorchis felineus, 357
Opisthorchis viverrini, 357
Opportunistic infections/infestations, 346–347
Oral contraceptives. *see* Contraceptive steroids
Ovarian-type stroma, 215
Overlap syndromes, 81–83, 82f
Oxaliplatin, 282

P

Paediatric non-alcoholic steatohepatitis, 125–126, 126f
Panacinar necrosis, 96–97
Pancreas, immunohistochemical stains, 227t
Panlobular necrosis, 41–43, 96–97, 160
Paracetamol, 141, 141f
Paraquat, 150
Parasinusoidal cells. *see* Stellate cells
Parasitic diseases, 353–358
Parenchyma
changes, chronic hepatitis, 156–163
lobular lesion, 160–163
periportal lesion, 156–163
Parenteral nutrition, 139, 307–308, 307f
Partial nodular transformation, 214
Peliosis, 273, 274f
Peliosis hepatis, in AIDS, 347–348
Pembrolizumab, 138
Peribiliary glands, 214–215
Pericellular fibrosis, 124f
Periductal fibrosis, 195
Periodic acid–Schiff (PAS)-positive material, 15t–16t, 267
Peripheral γ-δ/αβ T-cell lymphomas, 236–237
Periportal lesion, 156–160
Perisinusoidal cells. *see* Stellate cells
Perisinusoidal fibrosis, 54, 54f
Perivenular necrosis, 390–391
Perls' method, 15
Peroxisomes, 418
PFIC. *see* Progressive familial intrahepatic cholestasis (PFIC)
Phenylbutazone, 138
Phospholipidosis, 137t, 140–141
Phosphorus toxicity, 137
Piecemeal necrosis, 43–44
Pigmented hepatocellular adenoma, 208–209, 210f
Pigments
abnormal macrophage, 56
ceroid, 141, 276–277
haemosiderin, 196
hepatocellular, 25–27, 26t, 27f
lipofuscin, 27f
Tan Kupffer cell, 56
Pit cell, 422
Plasma cell-rich rejection, 394–395
Plasma cells, 55, 398–399
in autoimmune hepatitis, 170–173, 171f
in chronic hepatitis, 169–170
Plectin, 310
Pleomorphism, 191
Pneumocystis carinii, 346–347
Polyarteritis nodosa, 267, 268f, 361
Polycystic disease, 299
Polymorphonuclear leucocytes. *see* Neutrophils

Polymyalgia rheumatica-giant-cell arteritis syndrome, 361
Porphyria cutanea tarda (PCT), 335, 365
Porphyrias
  liver in, 365
  systemic disease, 365
Portal changes
  acute viral hepatitis, 94
  chronic hepatitis, 156
Portal fibrosis in alcoholic, 131f
Portal hypertension, 270–273
  non-cirrhotic, 271, 272f
Portal inflammation, 383
Portal tracts, 206–207, 211–212
  in chronic hepatitis, 156
  in cirrhosis, 206–207, 219
  normal liver, 20–21, 21f
  vacuoles in, 139
Portal veins, 269–273
  herniation, 271
  in non-cirrhotic portal fibrosis, 271
  occlusion as cause of infarcts, 267
  in schistosomiasis, 270
  thrombosis, 270
Postinfantile giant-cell hepatitis, 343
Postinfantile giant-cell transformation, 161, 162f
Posttransplant lymphoproliferative disease (PTLD), 395–396, 396f
Pre-eclampsia/eclampsia, 366–367, 368f
Pre-existing liver disease, 366
Pregnancy
  acute fatty liver of, 366–367, 367f
  HELLP syndrome, 366–367
  intrahepatic cholestasis of, 368
  liver in, 365–368
  pre-eclampsia/eclampsia, 366–367, 368f
Premature ductopenic variant, 76
Presence of any related tissue damage, 326
Primary biliary cholangitis, 73–83, 174, 212, 361
  chronic inflammation, 78f
  copper-associated protein, 80f
  differential diagnosis, 76–77
Primary biliary cholangitis (*Continued*)
  drug injury, 76–77
  epithelioid-cell granuloma, 76f
  interlobular bile duct, 75f
  lymphocytes, 77f
  lymphoid, 75f
  portal lesion of, 74–77
  premature ductopenic variant, 76
  progressive lesion of, 77–81
  scarring without nodule formation, 81f
  stage 3, 78f–79f
  stages of, 74, 74b
  transplantation for, 393–394
  viral hepatitis, 77f
Primary biliary cirrhosis (PBC). *see* Primary biliary cholangitis
Primary graft dysfunction, 379
Primary hepatic lymphoma, 236–237
Primary iron overload disorders, 326–331, 326b
Primary leiomyosarcoma, 349
Primary lysosomes, 417
Primary non-Hodgkin's lymphoma, 240
Primary sclerosing cholangitis (PSC), 68–73
  and autoimmune hepatitis, 173–174
  bile-duct dysplasia in, 72f
  in childhood, 172
  cholangiocarcinoma in, 71f
  differential diagnosis of, 72–73
  forms of, 68, 68t
  inflamed portal tract, 70f
  in large portal tract, 70f
  oedematous, 69f
  recurrence of, 394
  staging, 267
Processing of tissue, 13
Prochlorperazine, 149–150
Progenitor cells, 160, 211, 223
Progressive familial intrahepatic cholestasis (PFIC), 309, 310t, 311f
  type 2, 312f
  type 3, 312f
  type 6, 313f
Progressive lesion, 80–81
Protoporphyria, 365
Pseudoacini, pseudoglandular transformation. *see* Rosettes, hepatocellular
Pseudolipomas, 216–217
Pseudo-portal tracts (PPT), 210f
Pseudotumour, inflammatory, 217, 218f
Psoriasis, 146–147
Pylephlebitis, 269–270, 270f, 353
Pyrrolizidine alkaloids, 137t, 282

Q

Q fever, 342, 343f, 349

R

Reactivation of hepatitis B virus, 100
Reactive haemophagocytic syndrome, 360
Recurrence of disease after liver transplantation, 210
Recurrent disease, 391–394
Regeneration, 191, 275–276
  nodules, 190, 202, 267
Rejection Activity Index (RAI), 385
Renal transplantation, 400
Reporting, of liver biopsies, 7
Reticulin, 14, 15t–16t, 188–189
  in cirrhosis, 206–207
  collapse or condensation, 267–268
  in dysplastic nodules, 219
  in nodular regenerative hyperplasia, 211–212, 213f
Reye's syndrome, 306–307
Rheumatoid arthritis, 146–147, 273
  liver in, 361–362
Rhodanine, 15t–16t
Rickettsial infections, 349–353
Rosettes
  formation, chronic hepatitis with, 162f
  hepatocellular in hepatitis, 161, 162f, 170–172, 171f
Rough-surfaced endoplasmic reticulum, 415
Routine microscopy, 34–35
Rupture of liver in pregnancy, 267

S

Sampling variation, 277–278

Sarcoidosis, 76–77
granulomas in, 340–341, 341f–342f
liver biopsy, 4
Sarcoma, 240
Sarcoma botryoides, 240
Scanning electron microscopy, 423
Schistosoma japonicum, 355
Schistosoma mansoni, 355
Schistosomiasis, 270, 355–357, 357f–358f
Schönlein–Henoch purpura, 267
Schwannoma, 216–217
Scirrhous hepatocellular carcinoma, 221–223
Scleroderma, 361
Sclerohyaline nodules, 342
Sclerosed haemangioma, 215, 216f
Sclerosing carcinoma, 223
Sclerosing cholangitis, 267
Sclerosing hyaline necrosis, 129–130, 130f
Scoring system
in autoimmune hepatitis, 170, 173–174
of chronic hepatitis, 175, 177t, 178–181
Secondary biliary cirrhosis, 66, 66f–67f
Secondary iron overload disorders, 326, 326b, 331–335, 331f
Secondary lysosomes, 417
Sections, for microscopy, 13
Semi-quantitative scoring, of chronic hepatitis, 174–181, 175b–176b, 176t–177t, 178f–180f
Sepsis
bile ductular cholestasis in, 354f
liver in, 352–353
Septa, 211, 271, 280, 322–323, 322f
in cirrhosis, 206–207, 219
Septicaemia, cholestasis in, 60f
Shock, 141, 267–268
Short telomere syndrome, 214
Sickle-cell disease, 275–276, 275f
Siderosis, 326
in AIDS, 348–349
cirrhosis with, 334f–335f
Sinusoidal congestion, 390–391
differential diagnosis, 53, 53f
Sinusoidal obstruction syndrome (SOS), 281–282, 400, 401f
Sinusoids, 194, 280
dilatation of, 197, 273, 274f, 279f
in vascular disease, 282
hepatic, 273–276
lymphocytic infiltration, 168
periportal, 273, 274f
sinusoidal obstruction syndrome, 281–282
Small-cell dysplasia, 243–246, 247f
Small-cell liver-cell dysplasia, 218
Small-duct primary sclerosing cholangitis, 69
Small-for-size syndrome, 378–379
Smooth-surfaced endoplasmic reticulum, 415
Solitary fibrous tumour, 216–217
Solvent abuse, 142
Space-occupying lesions, changes near, 276
Space of Disse, 23–24
Sphingomyelin lipidosis, 305, 305f
Spirochaetal infection, 351–352
Spotty necrosis, 38–39, 160–161
focal necrosis *vs.*, 41f
Staging
chronic hepatitis, 174–181, 176b, 179f–180f
METAVIR staging system, 175, 176b
primary sclerosing cholangitis, 267
Stains
choice of, 14–15
chromotrope–aniline blue, 14
collagen, 14, 280
copper, 321, 325f
copper-associated protein, 321–323
elastic fibres, 280
glycogen, 321
orcein, 163–166
periodic acid–Schiff stain, 14
reticulin, 14–15, 280
rhodanine, 323
Timm's silver, 323
Victoria blue, 163–166
Steatohepatitis, 1–2, 145–146, 145f, 195–196
alcoholic, 14, 121–126
drug-related, 145–146
hepatocyte ballooning in, 116f
histological differential diagnosis, 129
Steatohepatitis (*Continued*)
non-alcoholic, 125–126, 270
pathological features of, 121–126, 121b
scoring system for, 127t
Steatosis, 139
in AIDS, 348–349
chronic hepatitis, 169
in chronic hepatitis, 161, 169, 176
drug-related, 139–140
focal fatty change, 111, 111f
grade of, 111–112
liver pathology, 361
macrovesicular, 108–112
causes of, 109b
fat vacuoles, 108f
with focal inflammation, 115f
swollen hepatocytes, 109f
microvesicular, 109f, 112–114, 137t, 139–140, 140f
causes of, 114b
didanosine-induced, 140f
drug-related, 139–140
non-hepatitic liver-cell damage, 139, 140f
with mild periportal fibrosis, 118f
mineral oil globules, 113f
non-hepatitic liver-cell damage, 139
periportal, 110f, 111
perivenular, 111
in posttransplant biopsies, 400
stellate-cell hyperplasia, 112, 113f
zonation of, 110f
Steatosis-activity-fibrosis (SAF) score, 128t
Steatoviral hepatitis B, 166
Stellate cells, 211
hyperplasia, 112, 113f, 146
Steroid hormones, 147
relationship to nodules and neoplasms, 209–210
Steroid-induced cholestasis, 147
Storage disorders, 300–301
Subcapsular necrosis, 5f
Subcapsular zone, 214–215, 214f
Submassive necrosis, 41–43, 43f
Syndromatic intrahepatic bile ducts, 293–294
Syphilis, 351

Systemic disease
amyloidosis, 362–365
bacterial infections, 349–353
collagen diseases, 361–362
fungal infections, 349–353
gastrointestinal disorders, 358–360
granulomas, 339–343
haematological disorders, 360
immune-complex diseases, 361–362
light-chain deposition, 362–365
non-specific reactive changes, 365
parasitic diseases, 353–358
porphyrias, 365
rheumatoid diseases, 361–362
rickettsial infections, 349–353
viral diseases, 343–349
Systemic lupus erythematosus (SLE), 267, 342
Systemic mast-cell disease, 237
Systemic sclerosis, 361

T

Talc, 168
Tamoxifen, 145–146, 145f
Tan Kupffer cell pigment, 56
Teratoid hepatoblastoma, 240
Thalassaemia, 333f–334f
6-Thioguanine, 282
Thrombosis, 390
hepatic artery, 267
hepatic vein, 276
portal veins, 269–270
Total parenteral nutrition (TPN). *see* Parenteral nutrition
Toxic oil syndrome, 212
Toxic-shock syndrome, 353
Toxoplasma gondii, 353
Toxoplasmosis, 353
TPN. *see* Parenteral nutrition
Transgastric liver biopsy, 5f
Transitional diseases, 81–83, 82f
Transjugular liver biopsy, 187
Transmission electron microscopy (TEM)
gene array, 424–426
gene sequencing, 424–426
immunoelectron microscopy, 422
immunohistochemistry, 423–424
Transmission electron microscopy (TEM) (*Continued*)
of liver biopsies, 408–411
molecular analysis, 424–426
scanning electron microscopy, 423
ultrastructural changes, 411–422
Transplantation
kidney, 1–2
liver, 1–2, 173
allograft biopsy, 379–380
assessment of donor liver, 378–379
bone marrow transplantation, 400
differential diagnosis, 397–400, 399t
graft dysfunction, 389–397
graft rejection, 380–389
liver-cell ballooning after, 381f
pathological lesions after, 380f
renal transplantation, 400
venous occlusion after, 281–282
Traumatic artefact, 7f
Triad of cellular rejection, 383
Trichrome, 15t–16t
Trimethoprim-sulphamethoxazole (co-trimoxazole), 140–141
Tropical splenomegaly syndrome, 270, 353–355, 355f, 360
TruCut needle, 187
Tuberculosis, 350–351, 350f–351f
Tubuloreticular structures, 421
Tumour necrosis factor (TNF), 423f
Tumours, 13
benign, 206–217, 238
of blood vessels, 205t, 216–217
cholangiocarcinoma (*see* Bile ducts, carcinoma)
cytopathological diagnosis, 205, 242–254
epithelioid haemangioendothelioma, 282
extrahepatic, liver changes in, 234–237
hepatocellular carcinoma, 217–219 (*see also* Carcinoma, hepatocellular)
inflammatory myofibroblastic tumour, 217
Tumours (*Continued*)
liver
classification of, 205, 205t
genomic changes associated with, 205, 206t
lymphangioma, 215–216
malignant, 217–233
mesenchymal and neural, 216–217
metastatic, 6f, 234–235, 235f, 252–254
neuroendocrine, 212
neurofibroma, 216–217
Turner syndrome, 341
Typhoid fever, 350
Tyrosinaemia, 306

U

Undifferentiated sarcomas, 240
Ureagenesis, 306

V

Valproate, 137t, 139–140
Vanishing bile-duct syndrome, 51, 149–150
in children, 194–195
drug-related, 149–150
Vascular disorders, 267–282
Vasculitis, 267, 278
Veins, 14, 269–273, 278–280
obstruction, 270, 273, 278–280
occluded in alcohol-related liver disease, 270
occluded in cirrhosis, 281–282
Veno-occlusive disease (VOD), 281–282, 281f
Venous congestion, 276–282
Venous outflow obstruction, 276–282, 279f–281f
Victoria blue, 15t–16t
Vinyl chloride, 137t, 146, 271, 273
Viral diseases
acquired immune deficiency syndrome, 344–349
cytomegalovirus infection, 343–344
evidence of, 196
infectious mononucleosis, 344
Viral hepatitis, 77f
bile-duct epithelium, 95f
bridging necrosis, 96f–97f
chronic hepatitis, 103

Viral hepatitis (*Continued*)
cirrhosis, 104
delta hepatitis, 100–102, 101f
differential diagnosis of, 103
fatal outcome, 103
hepatitis A, 97–99, 98f–99f
hepatitis B, 99–100
hepatitis C, 100, 101f
hepatitis D, 100–102
hepatitis E, 102, 102f
hepatocellular carcinoma, 104
hepatocellular damage, 90–91, 91f
histological variants, 94–97
inflammatory infiltrate, 92–94
liver transplantation, need for, 103
multilobular necrosis, 98f
pathological features, 89–97
periodic acid–Schiff, 93f
Perls' stain-positive granules, 94f
portal changes, 94
Viral hepatitis (*Continued*)
portal tract, 95f
reactivation, 100
recurrent, 380, 391
residual changes, 104f
resolution, 103
reticulin framework, 93f
scarring, 103
surviving hepatocytes, 90f
types, 89, 90t
Viruses
Epstein–Barr virus, 217
HIV, 173
Visceral leishmaniasis, 355
Vitamin A, 137t, 146
Von Meyenburg complex, 214–215, 229–230

W

Wedge biopsies, 190
Wedge liver biopsy, 211–212, 213f
Whipple's disease, 358–359
Wilson's disease, 174, 321–323, 322f–323f, 325f, 416f
fulminant liver failure in, 324f–325f
Wnt/β-catenin signalling, 8
Wolman's disease, 305

X

Xanthogranulomatous cholangitis, 69

Y

Yellow fever, 343
Yttrium 90 microspheres, 9f

Z

Zahn's infarction, 270
Zidovudine, 139–140
Zonation of the liver, 8

Information about COVID-19 and the liver

Other novel viral syndromes may require consideration, as exemplified by the recent global pandemic of COVID-19 infection. This corona virus (severe acute respiratory syndrome Corona Virus 2 or SARS-CoV2) infection has largely resulted in pulmonary disease, but abnormal serum liver tests (chiefly aminotransferases) are reported in over 40% of index patients in Wuhan, China, where the outbreak began.[1,2] The limited pathologic data as of this writing suggests that steatosis and mild lobular and portal inflammation may be seen pathologically,[3] although a recent biopsy from an infected individual at our institution suggested that accentuated hepatocyte apoptosis may be an important diagnostic distinction (**Fig. 1**).

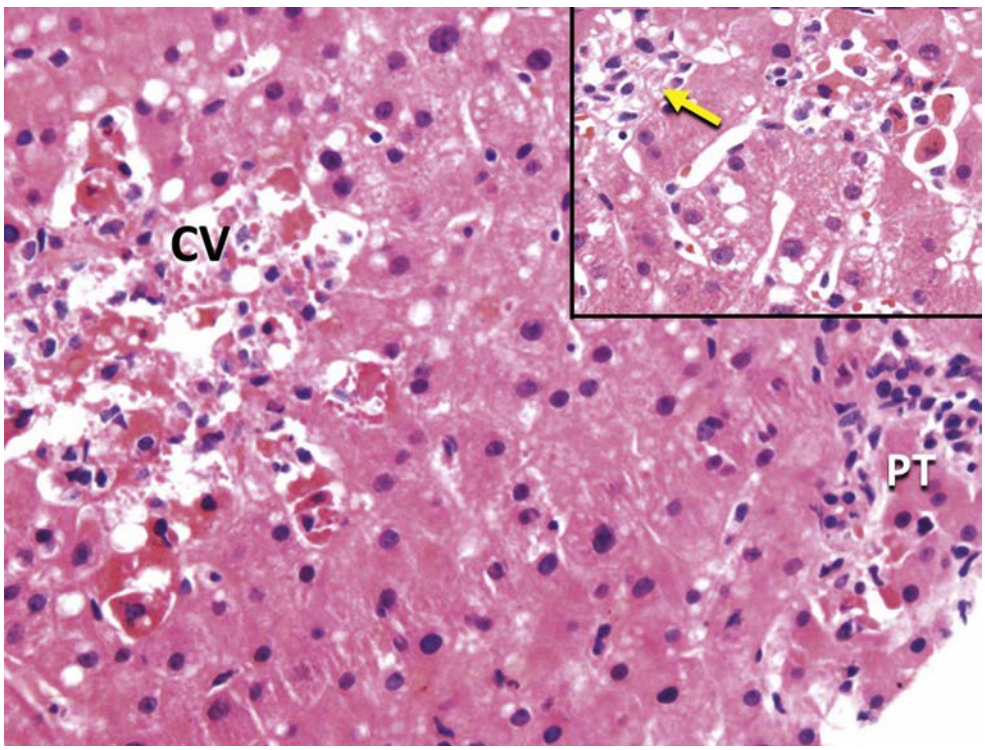

Fig. 1 Possible COVID-19 hepatitis one week after liver transplantation in an infant. Hepatocyte apoptosis and fragmentation are accentuated near the central vein (CV). The edge of a portal tract (PT) shows mild inflammation that was attributed to acute cellular rejection. **Inset:** Numerous lobular collections of apoptotic bodies are also present, with mild sinusoidal lymphocytic inflammation (yellow arrow). (Allograft needle biopsy, H&E)

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