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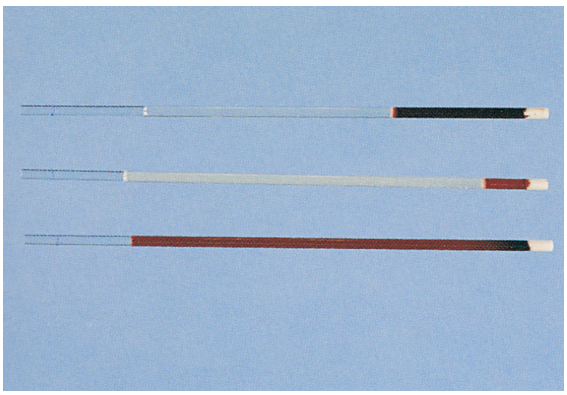
Preface

Our intent has been to help veterinarians and veterinary students appropriately select and accurately interpret laboratory tests in as simple, practical, and rapid a manner as possible. As with the first three editions, this fourth edition of *Small Animal Clinical Diagnosis by Laboratory Methods* is intended to present an organized method of answering commonly asked questions that reflect the problems frequently encountered with laboratory tests. Authors have updated their chapters as opposed to writing new ones with the idea that they could focus on making them as timely as possible. Tests that are less frequently used have been given substantially less attention or have been culled. The short section on serum biochemistry artifacts from the last edition has been expanded and a new author added. We have attempted to make this text as current as possible, but this goal borders on impossible in a profession that is continually advancing. As in prior editions, references and pathophysiology have been kept to a minimum because this text is designed to be user-friendly to both the busy clinician in the middle of a hectic day as well as the student that is just beginning to embark upon problem solving.

Acknowledgments

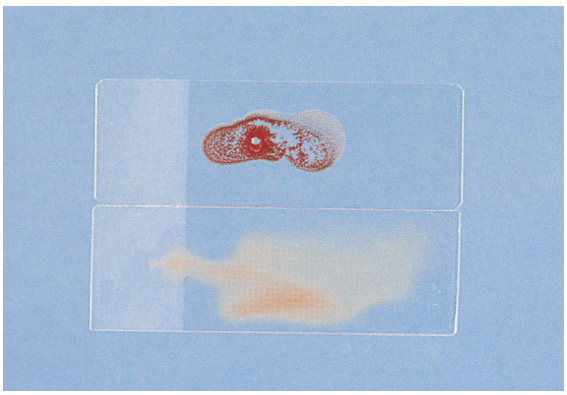
Each of us gratefully acknowledges the help, support, and tolerance of our families, friends, colleagues, lay staff, laboratory technicians, students, patients, other authors, and the universities we work with or have worked with, as well as the publishing staff of Elsevier. I (MDW) also wish to acknowledge the person who spoke the words recorded in Matthew 6:25-33 and John 10:10b. These verses sum it all up.

COLOR PLATES

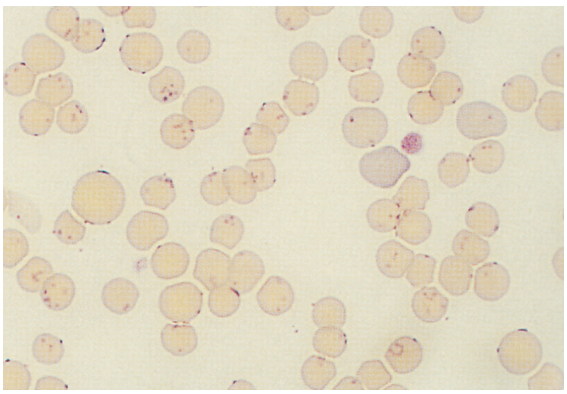


COLOR PLATE 1A. Three microhematocrit tubes with canine blood. The upper tube is normal. Both of the other tubes are from dogs with autoagglutination and immune-mediated hemolytic anemia. The middle tube has icterus, indicating extravascular hemolysis. The lower tube has hemolysis, indicating intravascular hemolysis.

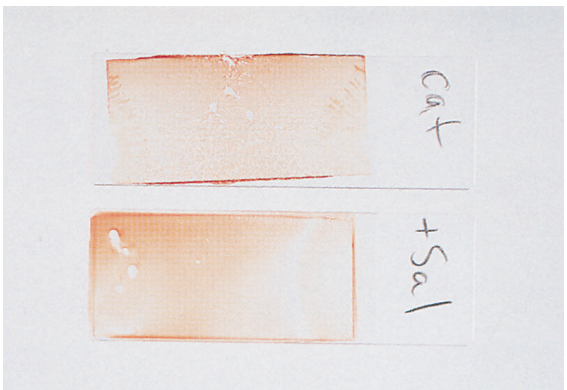
COLOR PLATE 1B. This drop of canine blood on a glass slide has autoagglutination of the red blood cells (RBCs) and icteric plasma.



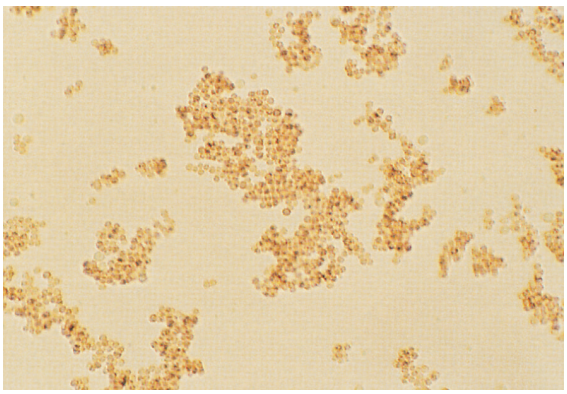
COLOR PLATE 1C. There are two drops of blood. The upper has RBC clumping from strong rouleaux of a severe inflammatory reaction *(Salmonella enteritis)*. The lower is blood mixed with saline. Rouleaux dissipates in saline, whereas autoagglutination does not.



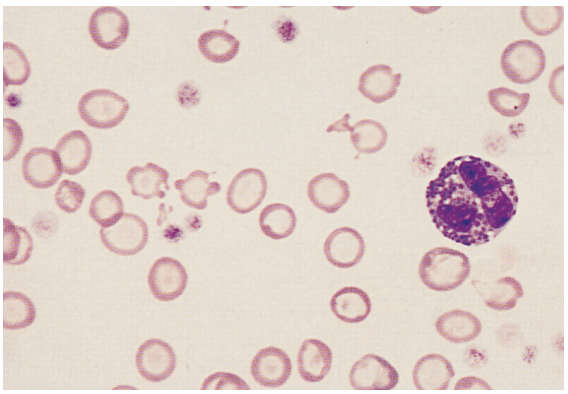
COLOR PLATE 1D. Blood smear from a cat with *Haemobartonella felis* infestation. Besides *H. felis* on the RBCs, there are two polychromatophils and one obvious macrocyte. See also Color Plates 1E and 1F from this cat.



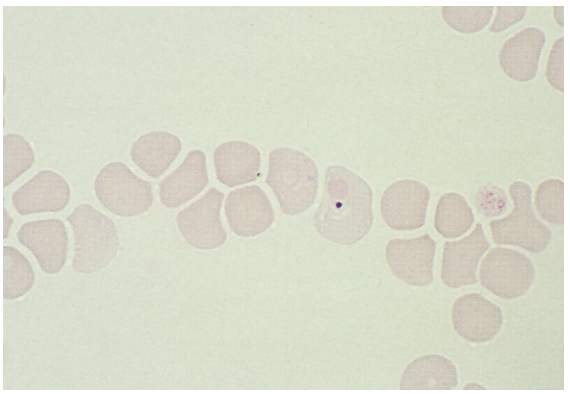
COLOR PLATE 1E. Gross appearance of blood on glass slides with coverslips from the cat with *H. felis* and immune-mediated hemolytic anemia in Color Plate 1D. The bottom drop was mixed with saline and the autoagglutination remained. The autoagglutination is much more subtle than the clumping in Color Plate 1B. See Color Plate 1F.



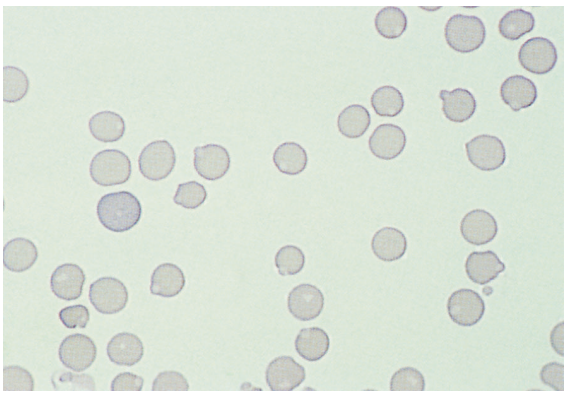
COLOR PLATE 1F. Microscopic appearance of the lower wet mount of blood and saline in Color Plate 1E. Clumping of RBCs remaining after mixing of blood and saline indicates autoagglutination.



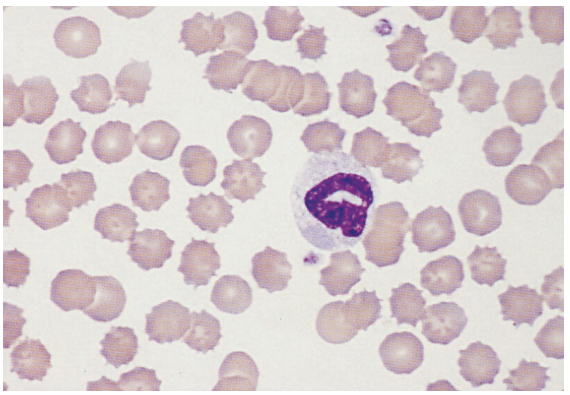
COLOR PLATE 2A. Canine iron-deficiency anemia. The erythrocytes have marked central pallor (hypochromasia) with only thin rims of hemoglobin. Erythrocyte fragmentation is also visible.



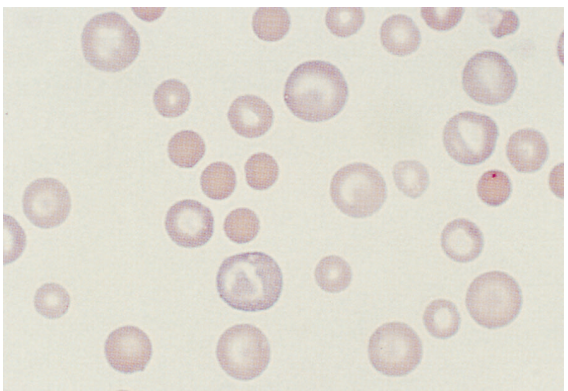
COLOR PLATE 2D. Canine distemper. The rounded viral inclusion bodies vary in color from gray to reddish. The most obvious inclusion body is above the dark blue, smaller Howell-Jolly body.



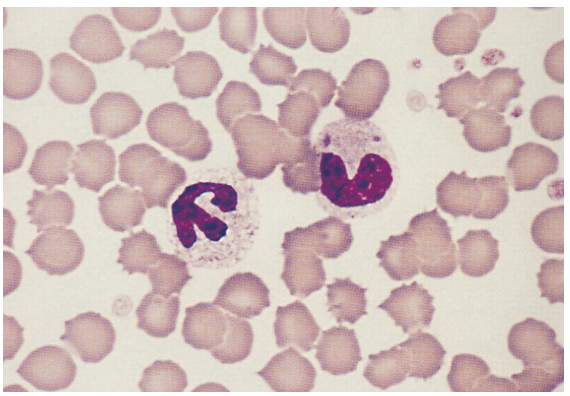
COLOR PLATE 2B. Feline Heinz body anemia. Many of the erythrocytes have a round, usually lighter staining Heinz body at the margin, with about half of the Heinz body extending above the surface. A free, round Heinz body has the same color as the hemoglobin in erythrocytes.



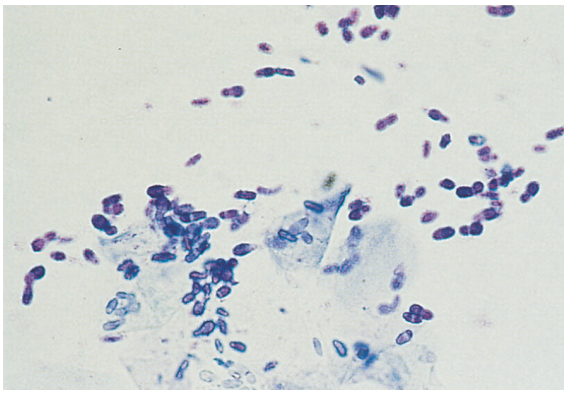
COLOR PLATE 2E. Canine distemper. There is a large, gray, viral inclusion body in the cytoplasm of the neutrophil at about seven o'clock at the cell margin.



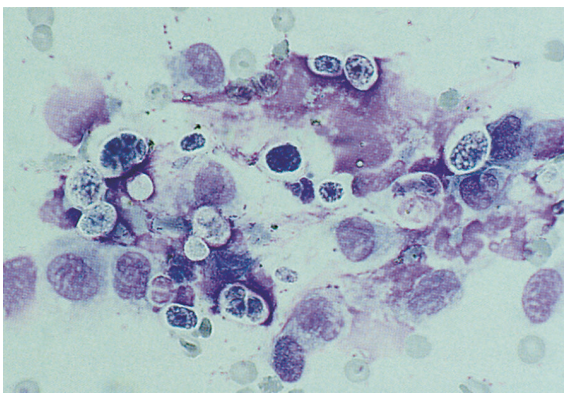
COLOR PLATE 2C. Canine immune-mediated hemolytic anemia. There is a cluster of four spherocytes, slightly up and left of center, and two spherocytes to the far right of center. Note that this is in an area where other erythrocytes show central pallor so it is easy to see the smaller diameter, darker color, and lack of central pallor in the spherocytes.



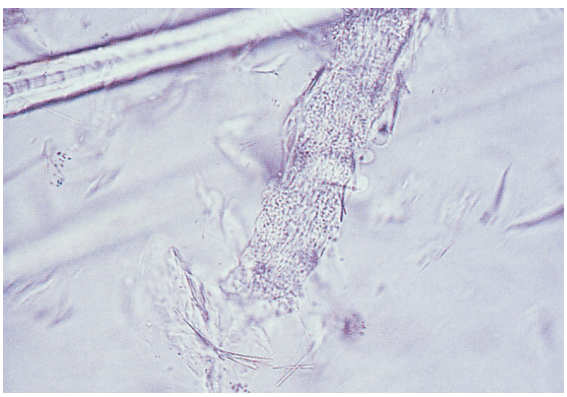
COLOR PLATE 2F. Canine toxic left shift. The bean-shaped metamyelocyte has two Döhle bodies at the poles of the nucleus. The segmented neutrophil has the foamy cytoplasm of toxic vacuolation.



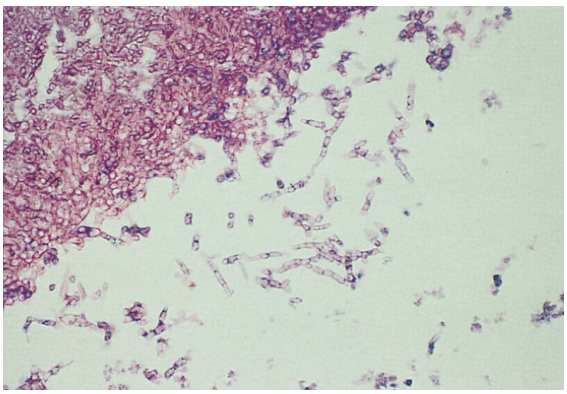
COLOR PLATE 3A. Canine yeast otitis. The ear swab had some thin squames and many small budding yeast to indicate a yeast otitis with *Malassezia (Pityrosporum)*.



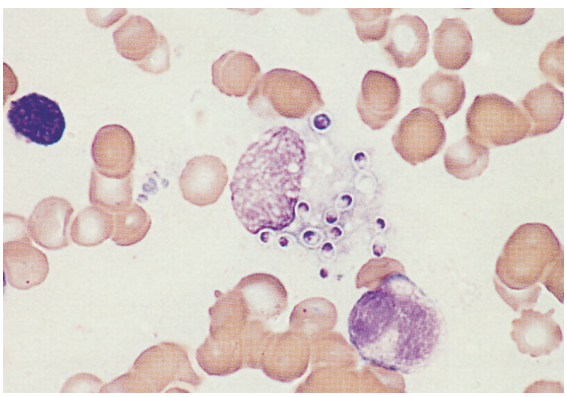
COLOR PLATE 3B. Canine rectal scraping. Besides inflammatory cells, the spherical organism with a clear halo are *Protheca*. (Courtesy of Dr. Rick Cowell.)



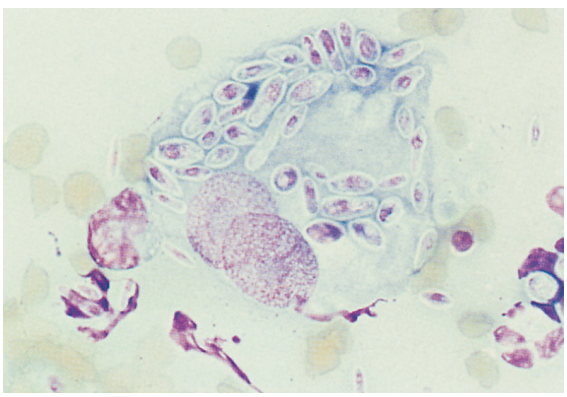
COLOR PLATE 3C. Canine ringworm skin scraping. One normal hair shaft is at the upper left. The swollen, fragmented hair shaft in the center is full of small, round *Microsporum canis* arthrospores.



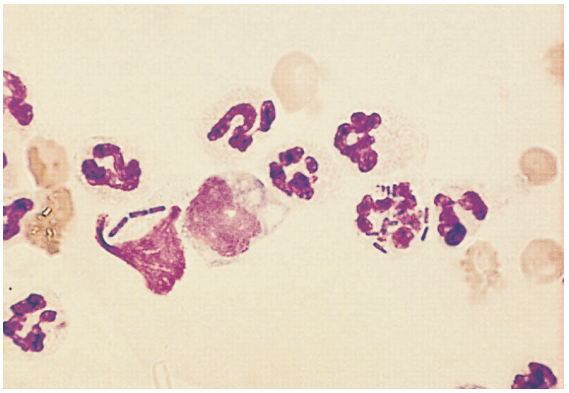
COLOR PLATE 3D. Nasal mycosis. A colony of *Aspergillus* consists of uniformly wide, septate hyphae. The smear of nasal exudate had only this one colony, which would have been missed if the smear had not been scanned under low magnification.



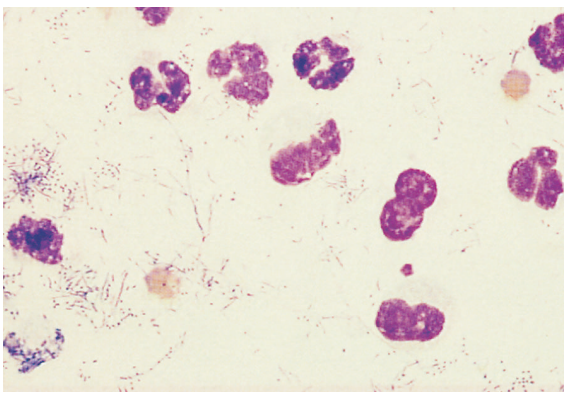
COLOR PLATE 3E. Canine histoplasmosis. A macrophage containing several small, oval, budding yeast with distinct cell walls was in an aspirate of bone marrow.



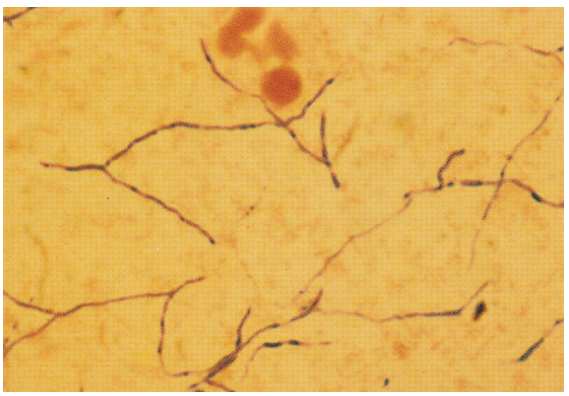
COLOR PLATE 3F. Feline sporotrichosis. The exudate from an infected declaw site had this macrophage filled with the pleomorphic yeast.



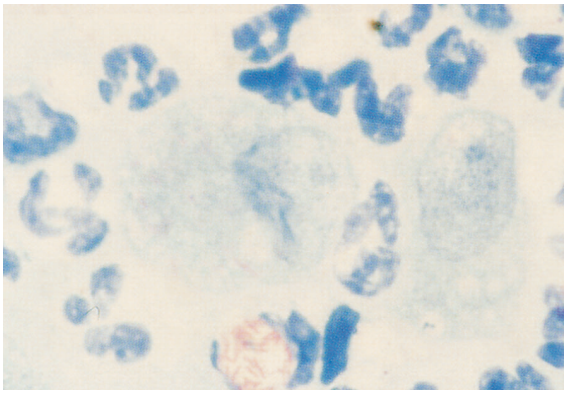
COLOR PLATE 4A. Septic exudate. Two neutrophils in this canine abdominal fluid contained rod-shaped bacteria. Note that the neutrophils do not look degenerate even though the exudate was septic. Do not consider the lysed neutrophil with bacteria to be degenerate.



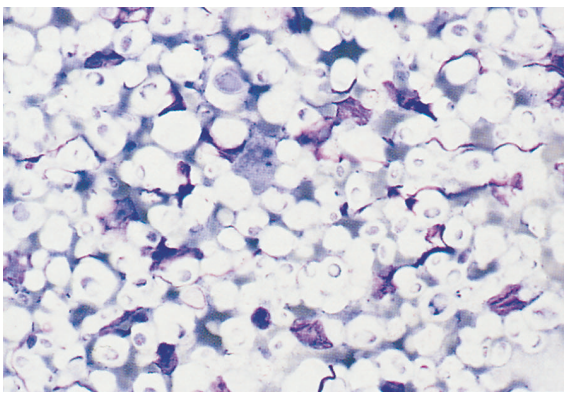
COLOR PLATE 4B. Degenerate neutrophils. The degenerate neutrophils in this thoracic fluid have karyolytic (swollen) nuclei as evidence of degeneration caused by the bacterial sepsis about them. The branching filamentous bacterium was *Actinomyces*.



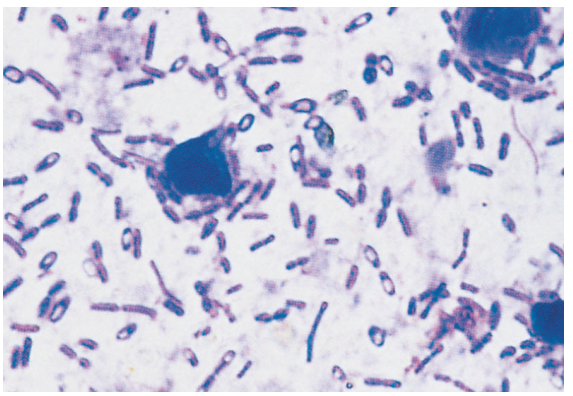
COLOR PLATE 4C. Gram's stain of exudate. The gram-positive, branching, beaded organisms were *Actinomyces*. Gram-negative organisms are difficult to find on gram's-stained smears of exudate. Many gram-negative rods are illustrated here, but often only one bacterium is found per several microscope fields in exudates and, if pale, a rare red bacteria would be easily hidden among red-staining leukocytes or debris.



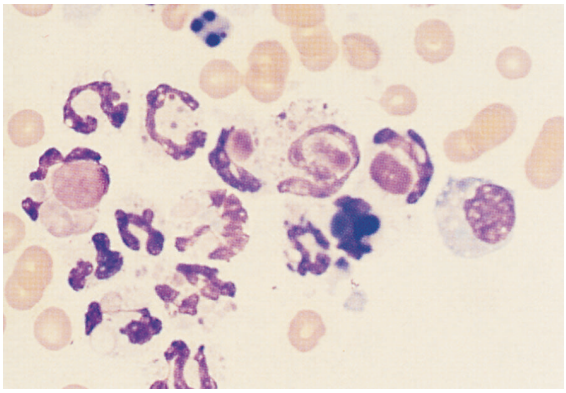
COLOR PLATE 4D. Acid-fast stain. The red, acid-fast positive rods of feline leprosy *(Mycobacterium)* are most obvious in a neutrophil at the bottom. A few rods were also in the two macrophages in the center.



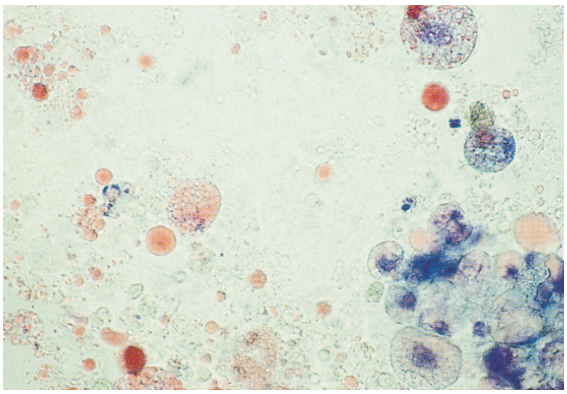
COLOR PLATE 4E. *Cryptococcus*. In this colony of *Cryptococcus* from the nose of a cat, note that the many yeast are surrounded by a large, clear halo representing the capsule. (Courtesy of Dr. Rick Cowell.)



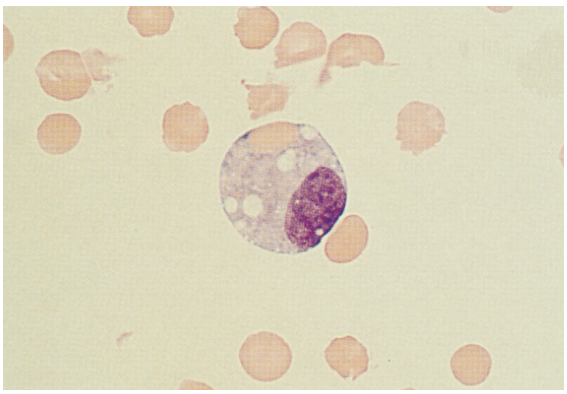
COLOR PLATE 4F. *Clostridium perfringens*. Smear of diarrheic feces with spore-forming bacteria indicating *C. perfringens*. The spores have clear spaces outlined by a dark wall.



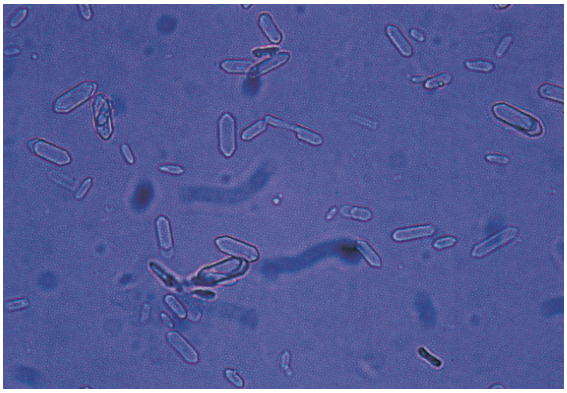
COLOR PLATE 5A. Canine lupus arthritis. This canine synovial fluid smear has one large lupus erythematosis (LE) cell at the far left, which is a neutrophil containing a large, round, violet LE body that is composed of nuclear proteins from a dead lysed cell bound to antinuclear antibodies. Many other neutrophils have multiple, smaller inclusions that are probably antibody-antigen complexes, and these white blood cells (WBCs) are called ragocytes. Both are indicative of immune-mediated joint disease.



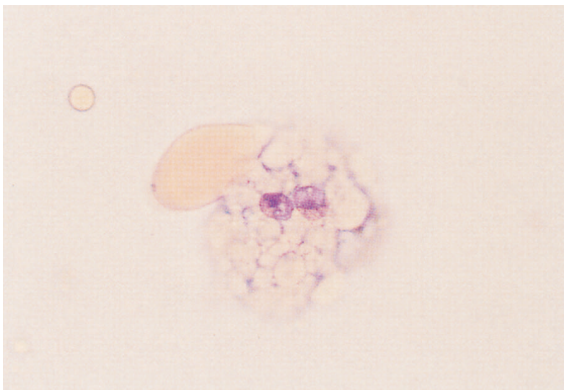
COLOR PLATE 5B. Chylothorax. A simple, cheap test for chylomicrons in thoracic fluid is to stain the smear with fat stain (e.g., Sudan stain or Oil-Red-O) to demonstrate neutral fat droplets in the phagocytes and free in the fluid, plus a drop of new methylene blue to stain the nuclei.



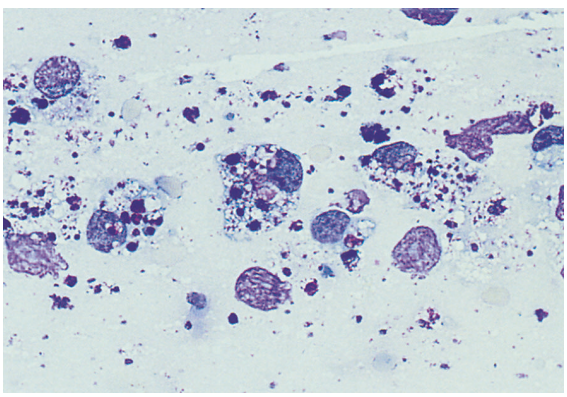
COLOR PLATE 5C. Pathologic hemorrhage. Erythrophagocytosis, such as in this macrophage, or hemosiderin in phagocytes indicates the hemorrhage in a fluid was, at least in part, from a pre-existing disease (i.e., pathologic hemorrhage) rather than an artifact of collecting the sample.



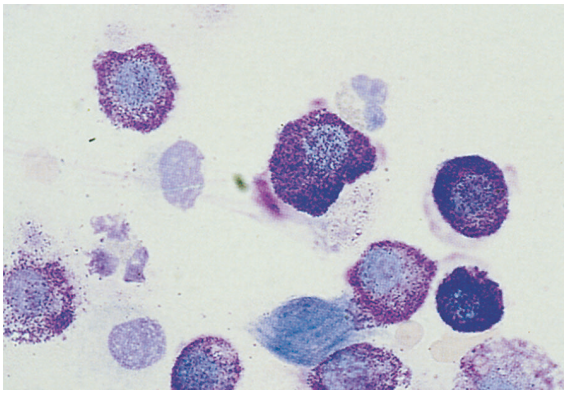
COLOR PLATE 5D. Calcium oxalate monohydrate crystals. These elongated six-sided crystals in urine of dogs and cats are highly indicative of ethylene glycol (antifreeze) toxicity.



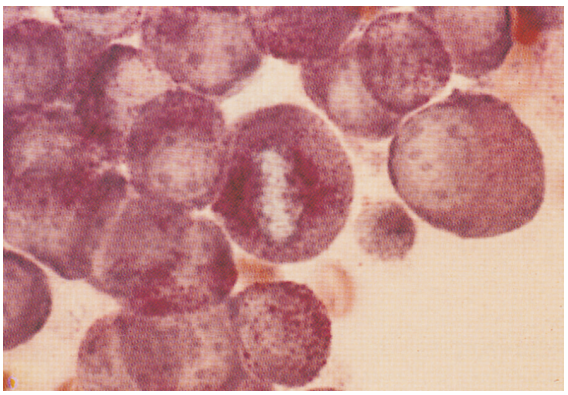
COLOR PLATE 5E. Feline fatty liver. This binucleated hepatocyte was stained with both a Sudan stain and new methylene blue to document the abundant fat in intracellular vacuoles and free outside the hepatocyte. Cytology is a rapid way to diagnose feline hepatic lipidosis though concurrent inflammatory disease may be hidden by hemodilution of the sample.



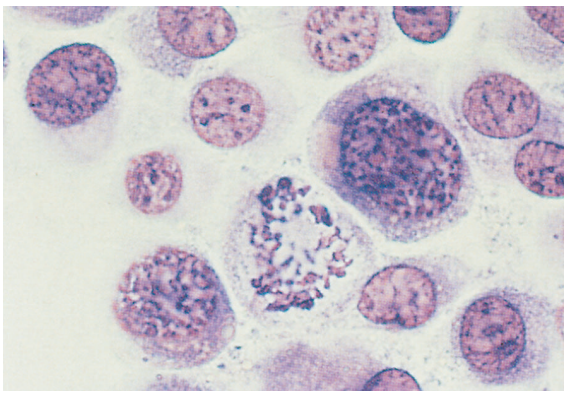
COLOR PLATE 5F. Injection site reaction. The smear of the aspirate has abundant debris free in the background and in macrophages. (Courtesy of Dr. Rick Cowell.)



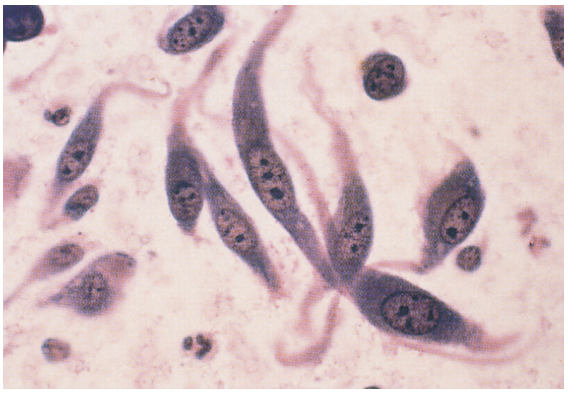
COLOR PLATE 6A. Canine mast cell tumor. Several well-differentiated mast cells are filled with uniform granules. Eosinophils are also visible.



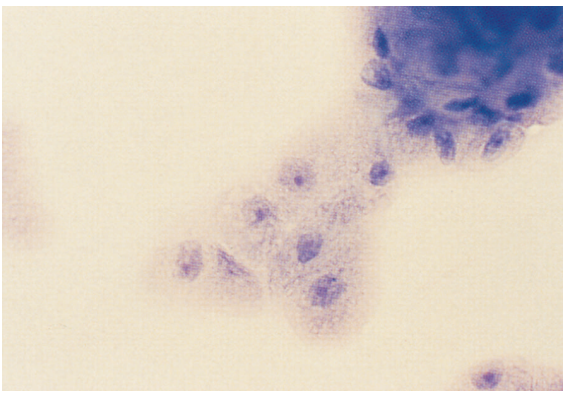
COLOR PLATE 6B. Malignant mast cell tumor. Compare with Color Plate 6A. Note the large nuclear size, multiple nucleoli, reduced number of granules, and mitotic figure.



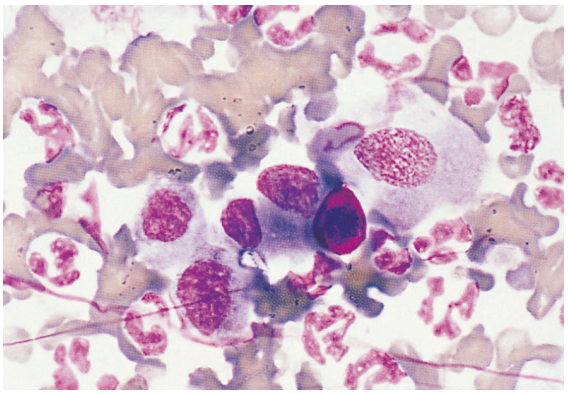
COLOR PLATE 6C. Canine malignant histiocytosis. Nuclear criteria are the strongest cytologic criteria of malignancy. Prominent variation among nuclei, especially in chromatin patterns and size, is strong evidence of malignancy.



COLOR PLATE 6D. Canine sarcoma. The spindle shapes of the cells indicate a connective tissue (mesenchymal) origin. Less obvious at this low magnification may be the large and variable nuclear and nucleolar size variation that indicated malignancy. Compare the size of the malignant nuclei with the small neutrophil at the lower center.



COLOR PLATE 6E. Perianal gland adenoma. The cells of this common neoplasm are easily recognized by their characteristic granular and abundant cytoplasm. Their resemblance to hepatocytes gives the neoplasm the name "hepatoid tumor."



COLOR PLATE 6F. *Rhinosporidium*. Besides the neutrophils and epithelial cells in the smear of a nasal mass in a dog, the reddish endospore of *Rhinosporidium* is just right of center. (Courtesy of Dr. Rick Cowell.)

1

Harold Tvedten and Jennifer S. Thomas

General Laboratory Concepts

LABORATORY TESTING CHOICES

Veterinarians have many choices regarding laboratory testing. Important factors include need and usefulness, practicality, cost-effectiveness, accuracy, and turnaround time. One must determine what tests to do in house and what tests to send out. Correct choices vary with the individual and situation, and no one answer fits all situations. Additional topics addressed in this chapter include statistics, quality control (QC), reference values, profiles versus individual tests, and international units (IUs).

To get a specific and meaningful answer, the reader must ask a specific and meaningful question and know whether a particular laboratory test is likely to yield a useful answer. As an example, the reader should compare the likely outcome of asking the following questions: Is the animal anemic? What is wrong with the animal? A microhematocrit procedure (in addition to knowledge of the animal's hydration status) will usually answer the first specific question, but a serum chemistry profile, complete blood count (CBC), urinalysis, and fecal examination may or may not answer the second vague, nonspecific question. One clinician should ask, "What will a high, low, or normal test result specifically mean in terms of a change in treatment or diagnosis?" If the answer is meaningful (i.e., it will change some action taken by the clinician), the test is worth the cost. Normal laboratory results may eliminate certain diseases and can be as valuable as abnormal results. See discussion of laboratory definitions and laboratory conclusions in Chapter 2.

> NOTE: To get a good laboratory answer, a good question should be asked.

SIMPLE STATISTICS

A reasonable level of skepticism about laboratory results should be maintained. *Clinicians should not believe all numbers*. Unexpected results are common and may necessitate reevaluating a provisional diagnosis. Alternatively, they may be erroneous. Laboratories should be asked to recheck unexpected results. One may need to repeat the analysis with a fresh sample. Trends over several days are often more informative than test results on a single day. Typically, not all test results that "should be" abnormal in a disease situation are abnormal in each affected patient.

NOTE: A reasonable level of skepticism about laboratory results should be maintained.

A few basic comments and the following examples illustrate interpretation of certain laboratory tests. Reference intervals represent 95% of normal animals. Thus 5% of tests (i.e., 1 of 20) are outside the reference interval. If a profile of 20 tests with 95% specificity for each test is used, only 36% of normal animals will likely have normal results on all 20 tests (Gerstman, 1986). One must expect some false-positive and false-negative test results. No tests are 100% sensitive and 100% specific for a disease.

NOTE: Only slightly more than one third of normal animals are likely to have "normal" results in all tests of a 20-test profile. The clinician should not overinterpret small changes from reference values.

False-positive results are often only slightly abnormal. The magnitude of a change helps determine one's confidence that a disease is present. Large alterations usually allow greater confidence, because they are seldom the result of statistical chance. With many tests, increasing magnitude of deviation from normal reflects a worsening prognosis.

One should remember that tests have inherent error and variation. The error of a manual leukocyte or erythrocyte count can be 10% to 20% because of technique; therefore mild changes from one day to the next may reflect only variation in the procedure rather than actual changes in the patient.

Evaluating populations of apparently well animals is much different from testing individual sick animals. The predictive value of a screening test and the prevalence of disease in an area should be considered (Casscells, Schenberer, and Graboys, 1978). For example, if a disease occurs in 1 of 1000 animals and a test is 95% specific for the disease, *what is the chance that an animal with a positive test result actually has the disease?* Most students, residents, and clinicians answered this question incorrectly; the average response was 56% with a range of 0.095% to 99%. A test with 95% specificity yields 5% false-positive results. Thus 5% of 999 animals, or about 50, will have a false-positive test result. However, because only 1 in a 1000 have the disease, only 1 of those 50 animals with a positive test result (i.e., 2%) will have the disease. The number of false-positive reactions is much smaller when a disease is common in the area and the animal has clinical signs of the disease.

NOTE: Evaluating test results from populations of apparently well animals is much different from testing individual sick animals.

Screening for a treatable, serious disease in well animals is exemplified by heartworm testing. For example, if a screening test for heartworm disease is 90% sensitive and 98% specific is used in 100 dogs (Courtney and Cornell, 1990), and if the incidence of disease is 1%, then one should identify the one ill dog and obtain two false-positive results in well dogs. Using the screening test in this situation creates the problem of what to do with the two well dogs, which may be subjected to needless and potentially toxic therapy plus owner concern. If instead the 100 dogs were from a dog pound in which half the dogs had heartworm disease (incidence = 50%), the test should identify 45 of the 50 infected dogs and misdiagnose one healthy dog as diseased. The concern in this situation is the effect of not treating five diseased dogs with false-negative test results, and the potential for a toxic reaction in the one well dog.

Testing for feline leukemia virus (FeLV) in apparently well cats has been statistically evaluated (Romatowski, 1989). The positive and negative predictive values are indicators of the probable accuracy of positive or negative test results. Given an estimate of 2% of cats having FeLV and use of a test with 95% sensitivity and specificity, it was calculated that a negative result should be 99.9% accurate but a positive reaction would be accurate only 28% of the time. The concern here is how to handle cats with a false-positive result for an untreatable disease. It was estimated that it would cost cat owners about $900 for each truly positive result.

QUALITY CONTROL

Accurate results require a QC program, because all instruments, reagents, laboratories, and people eventually make errors. These problems must be detected and corrected as soon as possible. QC should be a *major* consideration

in laboratory testing but is often poorly performed in in-clinic laboratory testing.

Intralaboratory QC is performed by repeated analysis of control reagents containing known amounts of various substances (i.e., analytes) such as glucose or urea nitrogen. Results on control serum should be within the range of expected QC results for that particular serum. Control sera with high, low, and normal levels of each substance should be used. In laboratories with a large volume, all three control sera (i.e., high, normal, low) are analyzed with each batch of patient samples. Results for patient samples are not reported if control sera results are out of range. QC values of control serum are recorded daily to detect sudden shifts or gradual trends suggesting instrument, personnel, or reagent problems. Gradual trends may be the result of slow deterioration of a reagent or slowly decreasing light intensity in an instrument. Rapid shifts may be the result of introducing a new reagent, a sudden change in an instrument component, or human error.

External QC programs document that procedures are comparable to those of other laboratories using those procedures. Veterinary laboratories are not legally required to participate in external QC programs, but they benefit from external QC programs. The College of American Pathologists (CAP) program is high quality, expensive, and required by laboratories testing human samples. Larger veterinary laboratories may purchase the program. Laboratories in these programs receive test samples, analyze the samples, and report their results. The performance of each laboratory in the testing program is compared with that of others using the same test method. Other external QC programs are available (e.g., from reagent or instrument producers). A practitioner can check a referral laboratory's precision by submitting duplicate samples to that laboratory or by submitting split samples to two or more laboratories to test accuracy.

REFERENCE VALUES

Reference values (i.e., reference ranges, reference intervals, means, "normal" values) are needed to judge whether a test result appears normal or abnormal. A laboratory result is meaningless without knowing what values normal animals in that situation should have. Reference ranges should optimally have confidence intervals around the upper and lower values but usually do not in veterinary laboratories. What are usually available are a range, mean, or both.

One uses the mean or range of reference values in different situations. Reference ranges are best for individual patients without previous evaluation. The best reference values are a patient's own values (if available) before an illness because individuals or members of special groups may have unique characteristics. When comparing groups of animals (e.g., a research project), one should use a mean value (e.g., packed cell volume [PCV] 45%) instead of a published reference range (e.g., PCV 37% to 54%).

Mean values also help detect trends from normal. This is important in interpretation of acid base values for example. If a dog has a low carbon dioxide partial pressure (PCO_2) (i.e., respiratory alkalosis) and a low bicarbonate (HCO_3) (i.e., metabolic acidosis), a pH still within the reference range can still be discriminating if it deviates from the mean. For example, a low-normal pH indicates an acidifying tendency and that the disease process is more likely metabolic acidosis with respiratory compensation, rather than respiratory alkalosis disease process with metabolic compensation. Means are used with increases in enzyme activity that should be reported as an *n*-fold increase (e.g., a tenfold increase over the mean).

Sources of reference values are often suboptimal. The expense is considerable considering the number of species involved; the variety of breeds; the effect of age, sex, and other factors; and the number (more than 120) of "normal" animals optimally needed in each category to establish reference values. Readily available sources of samples for reference ranges may be unsatisfactory. One attempt to establish hematology reference values at Michigan State University (MSU) used blood from microfilaria-negative samples submitted for heartworm tests. This sample pool, however, had several dogs with strong eosinophilia caused by probable occult heartworm disease, so it had to be discarded. Apparently normal dogs used for an MSU chemistry reference range, in retrospect, included several samples from a kennel with a subclinical, hereditary hepatic disease that skewed upward several hepatic test results.

New reference values should, theoretically, be established whenever a laboratory changes instruments or types of reagents, but the

expense may be prohibitive. For example, it took an $11,000 grant plus part of the time of three people working 4 months to establish new hematology reference values with a new hematology instrument for four animal species in 1988. At least 100 clinically normal animals of each species were bled, analyzed, and statistically evaluated. These data included only adult values and usually only one breed per species, so many variables, such as age, sex, environment, and breed, were not evaluated. One can extrapolate the amount of time and effort needed to maintain current reference values for even one species (e.g., dogs) if each breed and the effect of age are considered.

Literature values are often used for many tests and situations so that each laboratory does not need to create its own. One example is the International Species Inventory System (ISIS) Physiologic Data Reference Values, July 1995, for zoo animals. Many of the species are uncommon, and only a few may be present in a state or country. The ISIS values were derived from normal animals at 65 institutions so that a reasonably sized database could be derived. Basic hematologic results are more consistent among laboratories and techniques than chemistry values (especially enzymes); therefore hematologic reference values established by one laboratory are often accepted by others. A hematology reference text with reference values for a wide variety of age, breed, and sex factors is useful for interpreting data from pups, kittens, or breeds with unique characteristics (Feldman, Zinkl, and Jain, 2000). aboratories should develop reference values for their most frequently requested tests and species, but test volume in some species (e.g., pet birds, zoo animals) might be too low to justify establishing reference values. Feline reference values are sometimes neglected because it is more difficult to perform venipunctures on cats.

An alternative to obtaining at least 40 (and preferably 120) normal animals for establishing reference values is to use hospital patient data to obtain reference values. These values are not from animals proven to be normal but are an inexpensive, readily available source of a data pool large enough to represent the variety in the laboratory's patient population. Statistical manipulations produce cost-efficient reference ranges representing the hospital's population and the laboratory's current instrumentation.

The reference values in Tables 1-1 and 1-2 were derived by manipulating patient values.

TABLE 1-1. Canine Automated Hematologic Reference Values

	UNITS	MEAN	SD	REFERENCE RANGE
WBC count	1000/µl	10.46	2.7	6.4-15.9
RBC count	10^6/µl	6.69	0.6	5.6-8.0
Hemoglobin	g/dl	16.4	2.3	13.3-19.2
Hematocrit	%	45	4	36-54
MCV	fl	67.7	4.2	60-75
MCH	pg	24.4	1.6	21-27
MCHC	g/dl	36.1	1.6	34-38
Platelets	*10^3	345	101	186-547
MPV	fl	7.31	0.9	5.8-9.2
RDW	%	13.5	1.1	12-16
HDW	g/dl	1.94	0.3	1.6-2.7
Neutrophils*	%	71	11.5	43-88
Lymphocytes*	%	15	8.4	2.8-36
Monocytes*	%	5.0	2.4	2-11
Eosinophils*	%	4.4	5.1	0-17
Basophils*	%	0.3	0.2	0-0.3
LUC*	%	4.2	2.0	2-9

NOTE: Temporary reference values from patient results (365 dogs).
WBC, White blood cell; *RBC,* red blood cell; *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentration; *MPV,* mean platelet volume; *RDW,* red cell distribution width; *HDW,* hemoglobin distribution width; *LUC,* large unstained cell.
*Automated differential leukocyte counts, Bayer H-1 Hematology Analyzer.

TABLE 1-2. Feline Automated Hematologic Reference Values

	UNITS	MEAN	SD	REFERENCE VALUES
WBC count	1000/µl	10.9	3.6	6.6-18.0
RBC count	10^6/µl	7.5	1.2	5.3-10.0
Hemoglobin	g/dl	12.3	1.9	8.9-15.3
Hematocrit	%	36.3	6.0	25.8-48.1
MCV	fl	48	2.7	43.4-52.8
MCH	pg	16	1.3	14.1-18.6
MCHC	g/dl	34	1.9	30.6-35.8
Platelets	*10^3	404	92	300-631
MPV	Fl	10.5	1.6	8.5-13.2
RDW	%	16	1.4	14.0-18.1
HDW	g/dl	2.3	0.6	1.89-2.73

NOTE: Temporary reference values from patient results (33 cats).
WBC, White blood cell; *RBC,* red blood cell; *MCV,* mean corpuscular volume; *MCH,* mean corpuscular hemoglobin; *MCHC,* mean corpuscular hemoglobin concentration; *MPV,* mean platelet volume; *RDW,* red cell distribution width; *HDW,* hemoglobin distribution width.
*Bayer H-1 Hematology Analyzer.

These reference values included new hematologic parameters (e.g., hemoglobin distribution width, mean platelet volume [MPV]) that were unavailable in the literature. New reference

values were needed for clinicians to interpret these parameters until apparently normal animals could be obtained for more traditional reference values (see Appendix II). CBC data from patients were considered acceptable for determining the new reference values if the total leukocyte count, hematocrit, and platelet count were within previously reported reference ranges. The assumption was that if a patient had a normal leukocyte count, hematocrit, and platelet count, other, newer hematologic parameters would usually also be normal. Outliers were identified and removed. A 95% reference interval for each parameter was established by removing 2.5% of the lowest and highest values. The automated differential leukocyte count varies from what would be considered normal for a manual differential leukocyte count.

Another temporary alternative used for new techniques or instruments added to laboratories is to perform at least 40 duplicate analyses with the new and the previous or "standard" procedure and, by regression analysis ($Y = aX + b$), predicting the new procedure's range from the previous reference range. A key is to include a wide range of low and high results in the group of 40 or more duplicate samples. The regression analysis also validates that the new procedure, instrument, or reagents give comparable results.

Laboratory users should request the source of a laboratory's reference ranges. If one uses an in-office chemistry unit, one should establish one's own reference values for the instrument or validate a manufacturer's published reference range for the instrument. This is especially true with parameters such as enzymes, which vary the most from procedure to procedure.

PROFILES VERSUS INDIVIDUAL TEST SELECTION

Individual test selection or the use of a profile of tests has advantages and disadvantages. The choice is affected by the availability of tests, cost, turnaround time, and the problem to be evaluated. A well-designed profile should, for a minimal fee and minimal redundancy, have a high probability of detecting the common diseases for the particular situation. A CBC is a profile of many tests that screens for anemia, inflammatory disease, stress, thrombocytopenia, heartworms, and various other problems. The urinalysis is a profile of tests designed not only to reveal hemorrhagic, inflammatory, or functional deficits in the urogenital tract but also to detect diabetes mellitus, hepatic disease, massive acute muscle injury, or intravascular hemolysis. A hemostatic profile for evaluating bleeding disorders is discussed in Chapter 5. Serum chemistry profiles have variable numbers and types of tests included, although the "basic profile" includes the same or similar tests in most laboratories. Some criticize profiles for being a "shotgun approach" or a nonspecific "fishing trip." Profiles instead should be considered cost-effective screens for a large number of common problems.

Individual test selection becomes important if instrumentation requires one to perform each test individually or if certain tests are not offered in an inexpensive profile. Some tests (e.g., endocrinologic) may not be cost-effective enough to include in an initial screening profile. Tests that infrequently have abnormal results or that are expensive are requested when patient information indicates that a problem that justifies the use of more specific tests is likely. If a certain disease such as diabetes has been diagnosed, only one test (e.g., urinary glucose) may be needed to monitor the treatment process.

INTERNATIONAL SYSTEM OF UNITS

The International System of Units (Systéme Internationale dÚnites [SI units]) has standardized the reporting of data for improved comparison of results throughout most the world, with the exception of the United States, Brazil, and a few other countries. Units used for serum enzyme activity were particularly inconsistent in the past, when many enzyme procedures had results reported in units named after the author of the procedure. Now enzyme activity is reported as international units per liter (IU/L) in the USA or ukat/L in most other countries. Note that IU/L for enzyme activity is not an SI unit! The SI unit for enzyme activity is ukat/L. U.S. laboratories still use "traditional" units such as mg/dl. To convert a result from one unit (e.g., mg/dl) to another unit (e.g., mmol/L), see Appendix II for common conversion factors. Unfortunately, laboratories in the same hospital may report results using different units of measure, causing confusion for clinicians when interpreting those results. Veterinary clinical pathologists in the United States decided not to switch to SI units until

human laboratories (comprising a vast majority of U.S. laboratories) change.

STAT TESTS

Very short turnaround time between sample collection and the availability of results is important in some "stat" situations (e.g., designing fluid therapy for acid base and electrolyte abnormalities). Some instruments analyze plasma or whole blood, which saves the time (e.g., 30 minutes) required for blood to fully clot before harvesting serum. Unlike cost-efficient batches, stat tests are usually analyzed individually, making them expensive. Stat tests require laboratory personnel to interrupt their efficient work routines to perform single tests.

SEND IT OUT OR DO IT YOURSELF?

One must decide what testing to do "in clinic" and what to send out. The quality of in-clinic laboratories varies greatly. Tvedten currently works in a full-service laboratory open extended hours, with six employees in the laboratory serving a large private veterinary hospital with 25 small animal veterinarians. The laboratory is recognized as a major strength of that hospital. He has also worked in clinics where tests were performed incorrectly because all lacked the knowledge and time to perform them correctly.

If quality laboratory results are a goal, an investment must first be made in experienced medical technologists or equivalent and provide them adequate time to arrange good laboratory testing. It is best to have a single person in charge of in-clinic testing to decrease operator error. Technician time must be allotted for training, inventory and maintenance of reagents, equipment maintenance, trouble shooting problems, and performing repeated measurement on samples with suspected artifacts or results that are questionable.

The clinic must also accept responsibility for assuring that results are reliable. One person should be designated in charge of QC to ensure that results are accurate, repeatable, and properly reported. QC test results must be performed and recorded to prove validity of the answers sold to the client. Practices with low-test volume should analyze control serum with each patient's sample and for each type of test performed. This is a significant expense in time and money, so the frequency and extent of QC testing is often suboptimal and trust in one's test results fades.

Reagent strips that measure glucose concentrations provide reliable and accurate results in most patients; however, a few patients have significant differences in the results using the strips when compared with a reference method. (Cohn et al, 2000). Various kits are available for identification of various other substances (e.g., parvovirus in feces, ethylene glycol in urine, serum titers for various organisms). Some are discussed in other chapters. For a limited number of chemical analyses, dip-and-read test strips are available that do not require special equipment. Strips that measure BUN concentration (e.g., Azostix, Bayer Corporation) tend to underestimate concentrations in dogs and cats, and they will not identify a significant number of animals with elevated concentrations (Hill, Correa, and Stevens, 1994).

Microscopic analysis of cytology and hematology specimens provides many simple, rapid conclusions; therefore someone in a practice should learn and practice these techniques (see Chapters 2 to 5 and 16). Many valuable hematologic or cytologic results are derived from subjective observations (e.g., blood smear or cytologic smear evaluation) rather than instrument based observations. A good quality microscope is necessary. "Quick" stains (e.g., Diff-Quik) are easy and provide consistent staining characteristics of blood smears, bacteria and fungi, and cytologic smears. New methylene blue (NMB) is easy to use for urine sediments, cytology smears, and reticulocytes in blood (see stain and microscope discussions in Chapter 16). Air-dried smears of urine sediment, blood, or cytology smears should be sent to a clinical pathologist when one has any doubt about the diagnosis. Urine sediment structures adhere better to slides coated with protein (albumin or serum).

Cell counts in blood and other fluids have been made easy by the simplicity and consistency of diluting samples with Unopette containers.* Manual erythrocyte- and leukocyte-counting techniques are discussed in Chapter 2, and platelet counting is discussed in Chapter 5.

A refractometer is needed to determine urine specific gravity, plasma total protein, and total solids in various body fluids. Many small animals with possible renal failure yield

*Unopette, Becton Dickinson and Co, Rutherford, NJ.

urine sample volumes too small to allow the less expensive urinometer to determine the specific gravity. Only a drop of urine is needed to determine specific gravity with the refractometer, which is essential for evaluating renal function. Similarly, only the volume of plasma in a microhematocrit tube is needed for an accurate estimate of plasma protein. The veterinary TS Meter is a refractometer calibrated for cats and dogs.*

Most laboratories need two good quality centrifuges. A single-purpose centrifuge for microhematocrit tubes costing $660 to $3500 is needed to ensure consistent PCV determinations. A basic centrifuge for separating serum or plasma costs about $720. Serum or plasma may need to be stored in a refrigerator (4° C) or freezer (–20° C) before it is mailed to a referral laboratory. Freezers included in refrigerators and many home freezers may not maintain a temperature below –20° C.

EXPANDED IN-CLINIC VETERINARY LABORATORY

Some veterinary clinics perform most laboratory tests in house, because they want short turnaround times or they lack confidence in local laboratories. Greatly shortened time to receiving the test result and thus more rapid reaction to an animal's problem is the main advantage. Four other potential advantages to in-clinic biochemical testing are (1) improved client satisfaction and time management by eliminating the need to delay treatment, surgery, or both until results are reported by a referral laboratory; (2) minimization of preanalytic errors associated with transportation problems; (3) increased incorporation of biochemical profiles into presurgical screens, geriatric screens, and routine health maintenance exams; and (4) it is a source of additional clinic revenue (Metzger, 1996).

Large referral laboratories, however, offer less expensive testing, better QC, better accuracy, and a wider range of tests than do in-house laboratories. Many veterinarians purchase in-office chemistry units only to find that without frequent calibration and consistent use of controls, the results are untrust-worthy. Over the years, different instrument manufacturers have promoted various systems that disappeared (along with reagents) a few years later.

Most veterinarians should use referral laboratories as much as possible. Use of a good veterinary referral laboratory is analogous to referring orthopedic or internal medicine cases to a specialist who has trained in a specialty, has extensive experience in one field, has a high volume of one type of problem, and has the equipment to do the procedure well. Similarly a referral veterinary laboratory should have the personnel, equipment, sample volume, experience, and training to provide more accurate results at a lower cost.

NOTE: Veterinarians receive the best laboratory results from veterinary laboratories with experienced laboratory personnel, and they are advised to use available services before setting up their own in-clinic testing.

REFERRAL LABORATORIES

Referral laboratories specializing in laboratory analysis have the advantage of well-trained, experienced personnel. Veterinary referral laboratories understand problems related to animal samples better, but most chemistry procedures are well performed in human laboratories. Protein analyses may be exceptions giving inaccurate results. Canine hematologic samples usually are adequately handled because of similarities between canine and human blood, but feline blood has smaller red blood cells (RBCs), large platelets, and frequent platelet clumping to cause errors.

Referral laboratories usually have larger, sophisticated instrumentation that better detects sample and laboratory errors. Small, simplified instruments usually report a number without additional information to detect errors. Larger, automated hematology cell counters have graphic displays to illustrate errors in what is being counted. Analyses may be performed by more than one method (e.g., two types of total white blood cell [WBC] count), and if results for the same parameter do not match, a flag signals the operator to check that value. These more sophisticated instruments usually also provide a much broader range of hematologic data that is more precise, accurate, and rapid than is obtained by manual techniques or simple cell counters (see Chapters 2 to 5).

*H Reichert, Division of Warner Lambert Technologies, Inc, Buffalo, NY.

Referral laboratories offer a wider variety of tests, whereas the variety of tests a veterinary clinic may offer is limited by test volume. Offering a wider variety of tests means more reagent storage and increased likelihood that reagents will be outdated before they are used. *Outdated reagents should never be used*. Some referral laboratories have courier systems that pick up samples and return results (e.g., by fax) within hours, answering the need for rapid answers.

A key to effective use of referral laboratories is good communications. Laboratory personnel will explain how to submit samples properly. When an unsatisfactory sample is received by a busy laboratory or when instructions or sample labeling are incomplete, someone must either try to contact the busy veterinarian (who may not be available) or arbitrarily decide what to do with the specimen. It is important not to waste the effort used in obtaining the sample or lose a diagnostic opportunity that may not be available later. Many laboratories have prepared written information on submission procedures and reference values pertaining to their laboratories (see Appendix I, Listing of Referral Laboratories). Questions about sample handling and submission and routine charges should be directed to the medical technologist, clerk, or secretary who answers them daily. The pathologist's time should be used for questions that are interpretive, diagnostic, or of a policy nature.

COST ANALYSIS FOR IN-CLINIC ANALYZERS

In-office chemistry instruments involve many overlooked costs. Often salespeople discuss only the cost of the instrument and reagent vials or cartridges. The cost of validating the instrument, service contract, establishing reference ranges, QC testing, calibration, instrument depreciation, labor, lost interest (if the money for the instrument had been invested), and hospital overhead should be calculated in the lowest and highest possible sample volume situations. Before deciding to perform in-clinic biochemical analyses, one must determine if enough tests will be performed to cover equipment and reagent costs, as well as if the clinic can accept the extra work associated with an in-hospital laboratory. Time spent performing analyses will distract technicians from patient care. Other hidden costs include repeat testing on problem samples, disposable supplies, waste disposal, and optional and accessory equipment (Vap and Mitzner, 1996).

Volume of patient samples analyzed is critical in determining the cost per test (Table 1-3). The cost for one test (e.g., glucose, alanine aminotransferase [ALT]) using an in-clinic chemistry analyzer may vary from $3.00 a test (if 10 tests are analyzed per day) to $17.50 (if three tests are analyzed per week). (Values come from a previous edition of this book [Tvedten, 1994].)

This should be compared with a serum chemistry profile from a local human medicine laboratory. For example, a $17.00 serum chemistry profile included two options: (1) ALT, glucose, blood urea nitrogen (BUN), blood gas (PCO_2, PO_2, pH, and calculated HCO_3), sodium, potassium, chloride, and total CO_2 or (2) calcium, phosphorous, potassium, triglyceride, cholesterol, ALT, aspartate aminotransferase (AST), BUN, serum alkaline phosphatase (SAP), total protein, gamma-glutamyltransferase (GGT),

TABLE 1-3. Costs of Stat Analyzers: Blood Gas and Electrolytes

	TEST VOLUME PER YEAR: IRMA		TEST VOLUME PER YEAR: NOVA 4		
	424	1728	424	1728	4000
Instrument cost	$6375	$6375	$23,900	$23,900	$23,900
Cost/test	$2.19	$0.54	$8.05	$1.98	$0.90
Service contract/year	$500	$500	$3610	$3610	$3610
Service contract/test	$1.18	$0.28	$8.51	$2.09	$0.90
Disposables/test	$0.95	$0.75	$3.51	$1.31	$0.75
QC reagents + controls	$1365	$1365	$1300	$1886	$2300
Reagents/year	0	0	$1500	$1731	$2200
Reagents/test	$6.75	$6.75	$3.54	$1.00	$0.55
Sample labor/test	$5.19	$5.19	$15.00	$11.10*	$6.00
QC labor/test*	$1.55	$0.36	0	0	0
Total cost/test*	$21.02	$14.65	$41.71	$18.48	$9.63

*See text for explanations.

and glucose. This is a cost of $1.20 to $1.70 per test. Profile fees at referral laboratories are low because of high-test volume and an incentive to attract customers. Individually analyzed tests are more expensive because the QC, time, and labor expense to do a batch of 10 to 15 tests is often little more than for one or two tests. Single ALT and BUN evaluations at a local laboratory cost $15.00 each, and blood gas analysis (always performed as an individual test) costs $22.00 (compare with Table 1-3). Electrolytes cost another $22.00.

One estimate for assigning hospital overhead costs to income-generating areas of a veterinary clinic was $50.00 per square foot of floor space. If a laboratory takes about 88 square feet, one should divide $4400 per year of overhead by the number of tests performed. If one performs five tests per day during 250 working days per year, or 1250 tests per year, the overhead per test would be $3.52. Income-generating areas must compensate for other areas (e.g., medical records, reception area, office space).

EVALUATION OF TWO BLOOD GAS AND ELECTROLYTE ANALYZERS

A comparison of two stat instruments that perform blood gas and electrolyte analysis demonstrates that blood gas and electrolyte analysis is practical and cost efficient for even small veterinary clinics or subunits of large hospitals. The Nova Stat Profile 4* is a typical blood gas analyzer with ion specific electrode technology for performing sodium, potassium, chloride, total CO_2, and ionized calcium analysis. It automatically self-calibrates frequently throughout the day, requiring a constant flow of reagents and gases through it. Medical technologists check accuracy daily with control reagents. With daily QC one can determine when electrodes or other parts need to be replaced. It is designed for high volume use in a central laboratory.

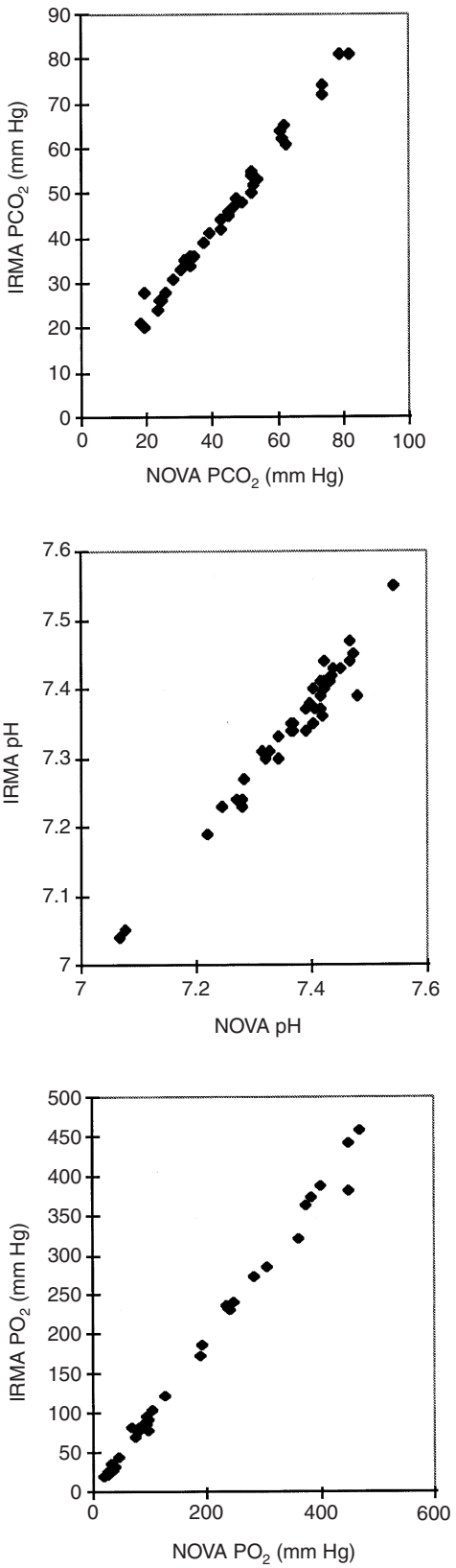
The IRMA (*I*mmediate *R*esponse *M*obile *A*nalysis)† is a point-of-care instrument in which electrochemical sensors are in disposable cartridges. The portable instrument heats the cartridge to 37° C, evaluates calibration data from the cartridge, reads patient results from the cartridge after whole blood is injected into it, and prints a report. An electronic QC system checks electronics in the instrument before each patient sample. Liquid QC reagents (i.e., high, normal, low) are analyzed each week to test three reagent cartridges for errors. Analysis can be performed at the side of anesthetized patients.

The IRMA was compared with a "standard" blood gas analyzer (Nova Stat Profile 4). About 40 patient samples with a wide range of normal and abnormal results were used in duplicate to determine if the IRMA's results were comparable to those of the Nova 4. Figure 1-1 shows excellent correlation over a wide range of results. The r-value for PCO_2 was 0.99. The IRMA averaged 1.3 mm Hg higher values. The r-value for pH was 0.98, and the IRMA averaged 0.03 unit lower values. The *r*-value for PO_2 was 0.997, and the IRMA averaged 8.1 mm Hg lower values (i.e., 155.1 compared with 166.2). Thus the IRMA's accuracy was considered essentially equivalent to that of the standard instrument.

A cost analysis is summarized in Table 1-3. The volume of tests per year were based on the Nova 4 analyzing 1728 tests in 1996 and the IRMA analyzing 212 tests in the first 6 months of 1997. Using the factors in Table 1-3, including instrument cost; service contracts; disposables such as syringes and paper for the IRMA or electrodes and plastic components for the Nova, QC reagents, and control reagents; labor for performing a test; and QC, the IRMA's cost per blood gas analysis at a volume of 424 tests per year was about $21.02. The Nova's costs for a blood gas analysis at a volume of 1728 tests per year was about $18.48.

These should only be considered approximations because of great variations in how one calculates costs. Life span of the instruments was estimated at 7 years, but many use a figure of 5 years. A shorter life span would increase per test costs. The Nova 4 was already 5 years old but in good working order. Labor costs per test used for the IRMA ($5.19) was 15 minutes per sample at the salary of an anesthesia technologist of $20.76 per hour. Labor costs assigned for every test in the laboratory had been set at $11.10. This was based on the total salary of all medical technologists in the laboratory for 1996 divided by the number of tests (hematology, chemistry, and manual procedures) performed that year. This includes all activities of the medical technologists, including QC, reporting, test development, instrument maintenance, vacation, and some periods of just waiting for samples. Using the salary of a medical technologist of $23.89 per hour, 15 minutes

*Nova Stat Profile 4, Nova Biomedical, Waltham, MA.
†IRMA, Diametrics Medical, Inc, St. Paul, MN.



would have cost $5.97. This plus labor costs for QC would have been less than the $11.10. Costs involved in the validation study (40 duplicate samples plus labor in evaluation) were not assigned to the IRMA. Hospital overhead and other potential expenses were not included. Thus different assumptions or approaches could have yielded significantly different costs.

In addition, the great variation in the cost per test is based on volume of tests performed per year. The 4000 tests per year for the Nova could easily be reached in a private laboratory emphasizing profit. By approximately doubling the volume, one might cut the cost per test about in half. Many such instruments are designed for high volume use. The decrease in cost per test for a point-of-care instrument such as the IRMA changed less, with about a fourfold increase in volume because of the relatively high cost per test of the disposable cartridges. The list price for these cartridges was $12.00 in 1996. The savings from point-of-care units are mainly seen in time, because one need not transport a sample to a central laboratory and wait for results or make a later effort to obtain results. In stat situations, treatment responses to changes in blood gas and electrolyte status can be more immediate. These estimates are for discussion only, but one may use similar approaches in determining one's own costs or deciding on buying an instrument.

Thus blood gas analysis can be performed in even small veterinary clinics for about the same expense ($18 to $22) charged by large referral laboratories. The IRMA provided accurate results and essentially immediate turnaround for immediate changes in treatment.

POINT-OF-CARE CHEMISTRY ANALYZERS

For tests that are truly stat (e.g., measuring blood glucose concentration for suspected hypoglycemic seizures), point-of-care testing is necessary. Point-of-care testing is performed in close proximity to the patient; it includes analyzers used at the patient's side and benchtop instruments. The ideal analyzer should be

FIGURE 1-1. The three scattergrams illustrate very good correlation among results for PCO_2 *(top)*, pH *(middle)*, and PO_2 *(bottom)* between a standard instrument, the Nova Stat Profile 4, and a newer point-of-care instrument, IRMA (*i*mmediate *r*esponse *m*obile *a*nalysis).

easy to use, provide fast and accurate results, require minimal operator time and training, be easy to maintain, have stable reagents, never break down, and be easy to repair. Published objective evaluation is available for relatively few commercially available analyzers (Grosenbaugh, Gadawski, and Muir, 1998; Lanevschi and Kramer, 1996; Sutton et al, 1999). Before purchasing or leasing a system, however, veterinarians should ask manufacturers to provide data showing the precision of their assays and how well the results compare with those obtained using a reference method for each species of interest.

When evaluating chemistry analyzers for purchase or lease, veterinarians should consider a number of other factors (Table 1-4). First, the number of different blood tests that an analyzer can perform should be determined and whether the range will meet the clinic's needs. The simplest analyzer measures the concentration of only one analyte (e.g., blood glucose). Advantages of blood glucose analyzers include rapid results, requirement for small quantities of blood (minimum requirement of 3 to 5 µl whole blood), affordability, and portability (Cohn et al, 2000). Evaluation of a variety of portable blood glucose meters (Glucometer 3/Glucofilm [Bayer Corp.], Glucometer Encore [Bayer Corp.], Accu-Chek Easy [Boehringer Mannheim], Exac-Tech RSG [MediSense Inc.], Glucometer Elite [Bayer Corp.], Glucometer DEX [Bayer Corp.], SureStep [LifeScan, Inc.], Precision QID [MediSense, Inc.], Accu-Chek Simplicity [Roche Diagnostics]) in cats and dogs indicate that they adequately predict the glycemic state of most patients and do not lead to erroneous treatment protocols (Wess and Reusch, 2000). However, the blood glucose concentrations reported by these portable analyzers are often different from those reported by a reference method, and these differences are most marked in hyperglycemic patients.

Many in-clinic chemistry analyzers measure multiple analytes (see Table 1-4). Those that allow the clinic to measure a single analyte or to customize a biochemical panel are the most flexible but tend to be more labor intensive and require more technician attention when performing a panel of tests. Analyzers offering a limited number of biochemical panels that are predetermined by the manufacturer are less flexible but generally cost less per test. They also tend to require less technician time and expertise (Vap and Mitzner, 1996).

Different chemistry analyzers often use different methodologies, therefore each analyzer system requires its own species-specific reference intervals that should be provided by the manufacturer. Reference intervals from referral laboratories should not be used for in-clinic chemistry testing unless the same analyzer system is used. Thus it is important when purchasing an analyzer to determine if reference intervals are available for the species of interest. Manufacturers should provide information about how the reference intervals were calculated (e.g., the number, age, breed, and sex of animals used) upon request. It is also important to know how in-clinic analyzers perform in ill patients, because the data provided by manufacturers are often established in healthy animals.

Cost and time required for QC procedures must be considered. QC involves measuring the concentration of analytes in known samples to detect analyzer malfunctions, reagent problems, and operator errors. Calibration refers to the procedures used to match analyzer output with expected results. Manufacturers should provide recommendations for a QC program. If a recommended QC program is not available or is inadequate, then that analytic system should be suspect. Some analyzer manufacturers recommend using liquid control solutions that mimic testing a patient sample; these evaluate the entire analytic process. Other manufacturers recommend electronic QC that uses a surrogate of some type instead of liquid controls to test analyzer function. Electronic QC alone may miss test reagent problems or some types of operator error (Ehrmeyer and Laessig, 2001).

Features of the analyzer operation are important. The ease of operation and training and sample size requirements should be determined. Analyzers that use small volumes of whole blood may eliminate some artifacts associated with plasma or serum separation. Analyzers that use plasma or serum require collection of 2 to 3 times more blood than the sample size required by the small-volume analyzer. One should also determine technician time required for sample setup and the time it takes to complete testing. Most in-clinic chemistry analyzers can provide panel results within 10 to 20 minutes; however, this time may not include analyzer or reagent warm-up times. Improper warm-up procedures can dramatically alter results. Finally, the linearity range of the assays

TABLE 1-4. Comparison of Some Commercially Available Chemistry Analyers

ANALYZER DISTRIBUTOR/ MANUFACTURER	ISTAT/HESKA	REFLOVET/PLUS SCIL	SPOTCHEMJ EZ HESKA	VET ACEJ/EXJ ALPHA WASSERMAN, INC	VETSCAN/ABAXIS, INC	VETTEST/IDEXX, INC
Number of available chemistry and electrolyte tests	8 plus blood gas and coagulation assays	14	19	25 (additional tests using reagents from other vendors) plus endocrine and drug assays	21 plus endocrine assays	21
Panels or individual test	6 predetermined panels + 2 individual tests	Individual tests	1 predetermined panel + individual tests	Individual tests + panels (customized by clinic)	9 predetermined panels	Individual tests + 3 predetermined panels
Species with available reference intervals	Dog, cat, horse	Dog, cat, horse, cow, rabbit, mouse, rat	Dog, cat, horse	Dog, cat	Dog, cat, horse, cow	>39 species & subspecies
Sample type	Whole blood	Whole blood, serum, or plasma	Whole blood, serum, or plasma	Serum	Whole blood, serum, or plasma	Serum or plasma
Sample size	100 µl	32 µl	250 µl (blood); 100 µl (serum/plasma)	50 µl (less with special) adapter	100 µl	95 µl
Recommended quality control procedures	Autocalibration/ EQC with each run; liquid controls as needed	EQC weekly; liquid controls as needed	Liquid controls once daily; calibrate with each new lot of reagents	Liquid controls once daily	Autocalibration/ EQC with each run; liquid controls as needed	Autocalibration/ EQC with each sample; monthly liquid controls
Reagent type	Cartridges	Dry test strips	Dry test strips	Liquid	Plastic rotors	Dry slides
Assay time	<2 mi	2-3 min per test	2.5 min for individual test; 7 min for panel	30 sec minimum for individual tests; panels <20 min	Panels <15 min	6 min for panel
Technical support*	24-hour support	Business hours; pager callback 24 hours	24-hour support	24-hour support	8 AM-8 PM EST Monday-Friday, 10 AM-4 PM EST Saturday	8 AM-9 PM EST; pager callback until midnight
Approximate analyzer list price†	$5495	$2995	$8500	NA	NA	$5000

*Current availability as of June 2002; the reader should check with manufacturer for any changes and for specific information about costs associated with technical support.
†Current as of June 2002; the reader should check with manufacturer for rental options, special discounts, and other specifics about analyzer and reagent costs.
EQC, Electronic quality control; *NA,* not available; *EST,* Eastern Standard Time.

should be determined. Results above this range are inaccurate and require dilution and retesting. Dilution of patient samples is time consuming and creates a source of operator error. Some analyzers provide automated sample dilution.

Reagent and equipment features are the final considerations. For reagents, the cost per test, shelf life, and storage requirements should be evaluated. If shelf life is short, reagents will probably become out-of-date frequently if the number of tests performed is low. Out-of-date reagents must be discarded and can be a significant expense. Equipment considerations include cost (e.g., purchase or lease, maintenance, service contract) and the time required for maintenance. If an analyzer malfunction occurs, one should determine the technical support offered by the manufacturer (e.g., times of day or week when available, cost), the expected turnaround time for repairs, and whether a loaner analyzer is available during the repair period. Because many of the analyzers currently available are computer driven, one should find out the expected frequency of upgrades and any associated costs.

Although reference is made to certain products and services (e.g., referral laboratories), readers should not favor one particular company's products discussed in this book or a particular referral laboratory listed in Appendix I over those unintentionally excluded. The products and laboratories discussed are those in the author's experience and are not necessarily better than those not mentioned. Costs change rapidly, so examples and quotes should be considered approximations.

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2

Douglas Weiss and Harold Tvedten

The Complete Blood Count and Bone Marrow Examination: General Comments and Selected Techniques

- **Complete Blood Count**
  - Magnitude of Hematologic Abnormalities
  - Frequency of Abnormalities
- **Quantitation Techniques**
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  - Microhematocrit
  - Hemoglobin Concentration
  - Total Cell Counts
  - Hemocytometer
  - Corrected White Blood Cell Count
  - Absolute Nucleated Red Blood Cell Count
  - Erythrocyte Indices
  - Automated Hematology Cell Counters
    - Impedance Counters
    - Laser Cell Counters
    - Quantitative Buffy Coat Vetautoread
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  - Stains
  - Evaluating Blood Smears
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    - Platelet Morphology
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    - Leukocyte Aggregation
    - Leukocyte Differential Count
    - Leukocyte Morphology
      - Left Shift
      - Toxic Neutrophils
      - Reactive Lymphocytes
      - Leukemia
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    - Erythrocyte Morphology
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      - Spherocytes
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  - Fibrinogen and Acute Phase Proteins
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  - Color of Blood
- **Bone Marrow Examination**
  - Hyperplasia and Neoplasia
  - Hypoplasia and Aplasia
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  - Acute Leukemia
  - Myelofibrosis and Necrosis
- **Bone Marrow Biopsy and Aspirate**
  - Evaluation of Bone Marrow Aspiration Smears
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    - Myeloid:Erythroid Ratio
    - Maturation of Myeloid and Erythroid Lines
    - Hemosiderin
    - Cellular Morphology
  - Summary of Bone Marrow Smear Evaluation Procedure

COMPLETE BLOOD COUNT

The complete blood count (CBC) is a profile of tests used to describe the quantity and quality of the cellular elements in blood and a few substances in plasma. The CBC is a cost-effective screen that detects many abnormalities and disease conditions. Bone marrow examination is used in selected instances to answer questions the more readily available CBC cannot.

How conclusions are derived from information in the CBC and bone marrow examination, in addition to selected hematologic techniques, follows. Explanations about interpretation are brief, because detailed discussions about the use of diagnostic tests for erythrocytes (i.e., red blood cells [RBCs]), leukocytes (i.e., white blood cells [WBCs]), and platelets are presented in Chapters 3, 4, and 5, respectively, and other references (Feldman, 2000). Technical comments are mainly restricted to common, basic tests; however, a few technically advanced procedures are included.

The CBC should be evaluated systematically. The first step involves identifying abnormal test results and using appropriate scientific terms to describe the abnormalities (Table 2-1). Adjectives such as mild, moderate, or marked reflect the magnitude of the change, which is important in interpretation.

Test results outside reference values for that species are usually considered abnormal. However, reference values are often not optimal. Reference values are usually derived from limited numbers of adult animals not segregated by age, sex, or breed (see Chapter 1).

TABLE 2-1. Definitions of Selected Hematologic Changes

HEMATOLOGIC CHANGE	DEFINITION
Anemia	Decreased red blood cell (RBC) mass, clinically noted by decreased packed cell volume (PCV)
Polycythemia	Increased RBC mass in body (increased PCV)
Polychromasia	Increased number of polychromatophils
Poikilocytosis	Increased variation in RBC shapes
Microcytosis	Increased number of small RBCs
Macrocytosis	Increased number of large RBCs
Normocytic	RBCs are of normal size
Hypochromic	RBCs have lower hemoglobin (Hgb) concentration (lower mean corpuscular Hgb concentration [MCHC])
Normochromic	RBCs have normal MCHC
Spherocytosis	Increased number of spheric RBCs
Echinocytosis	Increased number of RBCs with many spiny projections
Acanthocytosis	Increased number of RBCs with a few elongated, rounded projections
RBC fragmentation	Increased number of small RBC fragments, RBCs with extensions ready to break off, or both
Rouleaux	Formation of RBCs into linear formations resembling stacks of coins
Autoagglutination	Immune aggregation of RBCs into grapelike clusters
Heinz bodies	Precipitated Hgb resulting from oxidation
Thrombocytopenia	Decreased number of platelets
Thrombocytosis	Increased number of platelets
Leukocytosis	Increased number of white blood cells (WBCs)
Leukopenia	Decreased number of WBCs
Neutrophilia	Increased number of neutrophils
Neutropenia	Decreased number of neutrophils
Left shift	Increased number of immature neutrophils (nonsegs)
Right shift	Increased number of hypermature neutrophils (hypersegmentation)
Toxic neutrophils	Neutrophils with certain morphologic changes
Reactive lymphs	Lymphocytes with certain morphologic changes
Monocytosis	Increased number of monocytes
Monocytopenia	Decreased number of monocytes
Lymphocytosis	Increased number of lymphocytes
Lymphopenia	Decreased number of lymphocytes
Eosinophilia	Increased number of eosinophils
Eosinopenia	Decreased number of eosinophils
Basophilia	Increased number of basophils
Basopenia	Decreased number of basophils
Bicytopenia	Decrease in two cell lines (RBCs, WBCs, or platelets)
Pancytopenia	Decrease in three cell lines (RBCs, WBCs, and platelets)

These factors can be significant. For example, a reference range for canine packed cell volume (PCV) may be 37% to 55%. St. Bernards, however, tend to have PCV that range from 35% to 40%, whereas the PCV of greyhounds range from 52% to 60%. Prominent age related changes occur in dogs. At birth, RBCs are very large with a mean corpuscular volume (MCV) about 95 femtoliters (fl). The MCV decreases to adult values by 2 to 3 months of age. At 5 to 6 weeks of age, puppies normally have a PCV around 30%, a plasma protein around 5.3 g/dl, and 3% to 4% reticulocytes. These values could be confused with a regenerative, blood loss anemia if compared with adult reference ranges. Changes in laboratory methods cause minor to major differences in results. Reference values are costly and difficult to establish, so published values may be several years old. Thus one should interpret minor changes carefully.

The second step in evaluating abnormal results is to group together certain sets of data within the CBC. For example, a low PCV (i.e., anemia) should be linked to tests of bone marrow erythropoiesis, such as reticulocyte count and polychromasia, and observations on RBC morphology (see Chapter 3) to describe the anemia. A good description often leads to a good diagnosis. For example, a severe anemia with marked regeneration and many spherocytes and autoagglutination indicates clearly immune-mediated hemolytic anemia. Total leukocyte count should be linked with the differential leukocytes count and WBC morphology (see Chapter 4). A possible description may be severe neutropenia with more immature neutrophils than mature neutrophils, and marked toxic change, which indicates usually gram-negative infection or septicemia.

The third step involves drawing conclusions (laboratory diagnosis) from the sets of CBC results (Table 2-2). For example, severe anemia with marked regeneration, high normal plasma protein, moderate spherocytes, and autoagglutination is adequately diagnostic for immune-mediated hemolytic anemia (IMHA). Marked leukocytosis, neutrophilia, and moderate left shift without toxic change are commonly associated with IMHA in dogs and need not indicate that infection is present. The presence of lymphopenia is likely caused by stress or steroid treatment.

Magnitude of Hematologic Abnormalities

Magnitude of a change has great diagnostic significance and must be evaluated. Anemia in

TABLE 2-2. Hematologic Conclusions that May be Drawn from Hematologic Data

HEMATOLOGIC CONCLUSION	TYPICAL EVIDENCE
Regenerative anemia	Appropriate degree of increased reticulocytes or polychromasia for severity of the anemia
Nonregenerative anemia	Insufficient increase in reticulocytes for the severity and duration of the anemia
Hemolytic anemia	Strongly regenerative anemia with additional evidence, such as hemoglobinuria, normal to high plasma protein, and one of the causes of hemolysis
Blood loss anemia	Regenerative anemia with normal to decreased plasma protein, evidence of iron deficiency, or proof of blood loss
Immune-mediated hemolytic anemia (IMHA)	Moderate to marked spherocytosis, autoagglutination and/or positive Coombs' test
Oxidant-induced hemolytic anemia	Increased numbers of Heinz bodies, eccentrocytes or pyknocytes
Fragmentation anemia	Increased number of keratocytes, schizocytes, or acanthocytes
Iron deficiency anemia	Microcytic hypochromic anemia with variable regeneration
Inflammation	Leukocytosis, neutrophilia, left shift, eosinophilia
Stress or steroid reaction	Lymphopenia and eosinopenia; often neutrophilia, and occasionally monocytosis
Excitement/epinephrine response	Lymphocytosis, leukocytosis, neutrophilia, and perhaps polycythemia, especially in cats
Toxemia	Significant number of very toxic neutrophils
Myelodysplastic syndrome	Anemia or pancytopenia in blood and hypercellular marrow with dysplastic features
Acute leukemia	Significant number of hematopoietic blast cells in bone marrow and blood
Chronic leukemia	Neoplastic proliferation of mature appearing hematopoietic cells
Bone marrow disease	Pancytopenia or bicytopenia in blood; histologic and cytologic changes in bone marrow samples

ill animals is often mild. For example, in Table 2-3, 29% of 737 blood samples indicated anemia but 16 of the 29% (55% of the anemic dogs) had a PCV greater than 30%. Mild anemia is often secondary to a primary disease (e.g., anemia of inflammatory disease or hepatic failure), and one should mainly pursue the primary disease. Severe anemia (i.e., PCV < 20% in dogs), however, should be evaluated as a primary hematologic problem.

Frequency of Abnormalities

Numerous hematologic abnormalities are reported on CBCs (see Table 2-3). The frequency data were from two surveys of CBC results from consecutive patients at Michigan State University. The larger survey was a computer search of quantitative values. The smaller survey was a manual search of records that included subjective observations. One must be able to interpret which of these commonly reported results are clinically significant.

Anemia occurs frequently (10% to 29% of hospital patients), therefore the diagnosis of anemia needs to be understood (see Chapter 3). In dogs, anemia was most often regenerative as determined by the presence of polychromasia. Subjective observations obtained from microscopic evaluation of blood smears are commonly evaluated as 0 to 4+, with 0 indicating the change is absent and 4+ indicating a maximal increase. Abnormalities in RBC size (e.g., anisocytosis) and shape (e.g., poikilocytosis) are common reported (53% of feline CBC samples) but were often mild and clinically insignificant (e.g., 1+ anisocytosis, 1+ crenation). Anisocytosis indicates variation in RBC size, and 1+ is the smallest amount subjectively noted on a 0 to 4+ scale. Findings such as blood parasites, distemper inclusion bodies, or 2 to 4+ spherocytes are less frequent but are particularly diagnostic when identified.

Inflammation was moderately frequent in hospitalized dogs and cats, as demonstrated by a left shift (5% to 10%) and leukocytosis (13% to 28%) (see Chapter 4). The greater frequency of toxic change in feline neutrophils (23%) is due to the propensity of cats

TABLE 2-3. Frequency (%) of Selected Abnormalities in Two Surveys of Complete Blood Counts Performed at Michigan State University

ABNORMALITY	DOGS (N = 100)	DOGS (N = 737)	CATS (N = 30)	CATS (N = 159)
Anemia (PCV)	23	29	10	20
Anemia (Hgb)	13	29	10	21
Polychromasia	10	na	0	na
Abnormal RBC morphology	38	na	53	na
Microcytic (low MCV)	na	3	na	7
Hypochromic (low MCHC)	na	6	na	9
Macrocytic (high MCV)	na	5	na	7
Hyperchromic (high MCHC)	na	1	na	8
Polycythemia	5	3	7	9
Hypoproteinemia	9	na	7	na
Hyperproteinemia	24	na	47	na
Hemolysis	4	na	3	na
Lipemia	5	na	3	na
Leukocytosis	16	28	13	19
Leukopenia	na	6	na	22
Left shift	5	na	10	na
Lymphopenia	27	na	43	na
Lymphocytosis	2	na	3	na
Monocytosis	14	na	13	na
Monocytopenia	0	na	4	na
Eosinophilia	17	na	10	na
Neutrophil toxicity	4	na	23	na
Reactive lymphocytes	7	na	7	na
Thrombocytopenia	5	15	7	60
Clumped platelets	6	na	23	na
Thrombocytosis	5	11	7	6

PCV, Packed cell volume; *Hgb*, hemoglobin; *RBC*, red blood cell; *MCV*, mean corpuscular volume; *MCHC*, mean corpuscular hemoglobin concentration; *WBC*, white blood cell; *na*, not available because of design.

to form Döhle bodies (a very mild form of toxic change). Hyperproteinemia (47% of feline CBC) is frequently the result of inflammation, immune stimulation, or secondary to dehydration (see Chapter 12).

Low platelet counts (<200,000/µl) were common in feline samples (71%) but only 3% were true decreases (Norman, 2001b). Most were usually pseudothrombocytopenia caused by platelet clumping. Feline platelets tend to clump, which causes erroneous decreases in platelet count. Table 2-3 shows that the automated platelet count (Bayer H-1) was low in 60% of samples. Platelet clumping was reported in only 23% of the blood smears in the small survey of feline CBC (see Table 2-3), but this was probably that microscopists were so accustomed to clumping in feline samples that they reported only severe clumping.

QUANTITATION TECHNIQUES

Sample Submission

Anticoagulated blood is required for cell counts. Any visible clots in a blood sample eliminate any confidence in the WBC or platelet counts, because distribution of cells is not uniform in the blood. Clinical laboratories will not process clotted samples, because results are invalid and clots may plug hematology cell-counting instruments.

Ethylenediaminetetraacetic acid (EDTA) is the best anticoagulant to preserve cell detail. Heparin produces poor staining with a diffuse blue background. Formalin or formalin fumes cause poor staining of Wright's-stained blood smears, resulting in a blue background. One must keep formalin away from blood and cytology smears, both in the laboratory and in packages sent to referral laboratories. Formalin fumes can affect smears without direct contact. A citrate-based anticoagulant (Diatube-H, Becton Dickinson, Oxford, UK) is associated with good cell detail on smears (Norman, 2001a).

Commercial blood collection tubes contain a vacuum and should be permitted to fill until flow stops. Overfilling the tube may result in blood clotting, whereas, underfilling of the tube results in an excessive concentration of EDTA, which causes RBC shrinkage and decreases PCV. If very small amounts of blood are collected into liquid EDTA meant for larger volumes, dilution of blood may be significant, resulting in lower cell counts. Citrate blood collection tubes for hemostasis testing are marked with a line that shows the proper volume. Fill these to the line to avoid dilution or concentration errors.

If not analyzed within 2 to 3 hours, EDTA blood should be refrigerated at 4° C because RBC swelling after 6 to 24 hours of storage raises PCV and MCV and lowers mean corpuscular hemoglobin concentration (MCHC). The RBC count, hemoglobin (Hgb) concentration, hematocrit (Hct), and RBC indices (i.e., MCV, mean corpuscular Hgb [MCH], MCHC) have minimal changes if blood is refrigerated for up to 24 hours.

Blood smears should be prepared immediately and air-dried to avoid artifacts caused by exposure of cells to anticoagulants and cell deterioration during storage and shipment. For blood parasite examination, capillary blood (ear prick) is preferred for a higher concentration of parasitized RBCs. Anticoagulated blood from an ear prick is streaked out immediately on a blood smear.

Slides should not be mailed in the thin, cardboard mail cards that fit into envelopes. These envelopes are often machine processed by the post office, and the machines crush the slides. Instead, they should be mailed in rigid plastic containers (e.g., boxes) too bulky to be machine cancelled.

Microhematocrit

The microhematocrit method for determining PCV is routinely used to estimate erythroid mass. It is more consistent and technically easier than a manual RBC count or Hgb determination and provides additional useful information. Gross examination of plasma in the microhematocrit tube may detect icterus, hemolysis, or lipemia (see Color Plate 1A). The microhematocrit can also screen for heartworm disease, because microfilariae are concentrated in the plasma just above the buffy coat. Plasma protein concentration can be quantified using a refractometer on plasma obtained from one or two microhematocrit tubes.

PCV is determined by centrifuging anticoagulated blood in a microcapillary tube to separate cells from plasma. Microhematocrit tubes that contain heparin may be used for direct collection of nonanticoagulated blood. Microhematocrit tubes are filled to about two-thirds to three-fourths full. After centrifugation, RBCs are packed at the bottom and WBCs and platelets appear as a thin white line (i.e., buffy coat) between RBCs and

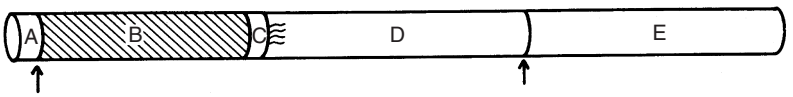

FIGURE 2-1. Microhematocrit tube. Arrows indicate the length of tube that contains blood. The packed cell volume (PCV in %) = B ÷ (B + C + D). The plasma area *(D)* adjacent to the buffy coat is where to check for microfilaria *(four wavy lines)*. *A,* Clay seal; *B,* packed erythrocytes; *C,* packed leukocytes and platelets (buffy coat); *D,* plasma; *E,* empty space in the top of the tube.

plasma (Figure 2-1). To calculate PCV, one divides the length of packed RBCs by the total length of the packed RBCs, buffy coat, and plasma. The clay plugging the bottom of the tube should not be included. Various microhematocrit reading devices are available.

Error in microhematocrit determination is minimal but, when present, is usually related to centrifugation. Microhematocrit tubes should be centrifuged for 5 minutes. When the PCV is greater than 50%, packing of RBCs by the centrifuge is less complete (i.e., less tight). This causes an overestimation of the PCV. If the microhematocrit tubes are filled to more than two-thirds to three-fourths full, cell packing is also less complete. When a PCV is less than 25%, the packing of RBCs is tighter. This exaggerates the decrease in the PCV and makes the animal seem slightly more anemic. Microhematocrit centrifuges attain high speeds (11,500 to 15,000 rpm), which ensures the proper centrifugal force to pack cells. The speed should be checked periodically, because slower speeds cause poorer cell packing that cannot be compensated for by longer periods of centrifugation. One should check the centrifuge's brushes three to four times per year and replace if worn. Microhematocrit tubes should be evenly balanced in the centrifuge's head to prevent unequal weight distribution. If the head is not properly balanced, the wear on the motor is uneven and eventually causes the head to vibrate. One should not use the brake when the head is still rotating at high speeds, because this also causes excessive motor wear.

Hemoglobin Concentration

Under most circumstances, Hgb concentration (i.e., Hgb), erythrocyte count (i.e., RBC), and PCV are essentially equivalent in estimating the animal's erythroid mass. Because the microhematocrit technique is simple, accurate, and inexpensive, most veterinarians determine PCV rather than Hgb or RBC. Hgb is obtained as part of the CBC performed by automated hematology instruments. Hgb may be more accurate than PCV if cell shrinkage, cell swelling, or increased cell fragility are present. Hgb is inaccurate if lipemia interferes with plasma light transmission during photometric analysis. Large numbers of Heinz bodies may cause an erroneous increase in optical density, thus artifactually increasing Hgb concentration.

Total Cell Counts

Total WBC, RBC, and platelet counts can be determined manually with a hemocytometer or with automated instruments. A hemocytometer is much less expensive and does not require calibration and quality control reagents. Automated instruments are much more precise and save time when one has multiple samples to analyze each day.

Total platelet counts are usually inaccurate in cats (see discussion of platelet counting in Chapter 5). Between one half to three fourths of feline blood samples have clumping of platelets to an extent that affects the platelet count. If prominent platelet clumping is observed, distribution of platelets in the blood sample is uneven and the accuracy of manual counts, automated counts, and estimates from the blood smear are adversely affected. Many laboratories perform a manual platelet count if too many or large platelet clumps are seen. However, the problem with inability to count accurately the platelets in the clumps remains. Manual counts include large feline platelets, which are often missed by automated instruments. One may also vortex mix (i.e., shake with a vortex mixer) the feline blood with platelet clumps to try to obtain a more accurate count. The platelet count increases after mixing but vortexing rarely eliminates all of the platelet clumping (Tvedten and Korcal, 2001). A trivial increase in the platelet count suggests thrombocytopenia was a disease change. Evaluation of platelet on a blood smear also may eliminate true thrombocytopenia if one sees large platelet clumps on the smear. Large platelet aggregates would not be expected in

true, severe thrombocytopenia. A citrate-based anticoagulant (Diatube-H, Becton Dickinson; CTAD) reduces pseudothrombocytopenia in feline blood samples. CTAD includes citrate, theophylline, adenosine, and dipyridamole, which inhibit platelet clumping better than EDTA (Norman, 2001a). These alternatives do not provide a consistently accurate feline platelet count but reduce the frequency and magnitude of the pseudothrombocytopenia.

Hemocytometer

A hemocytometer is a transparent glass chamber that holds a cell suspension for microscopic cell counting. The hemocytometer is 0.1 mm deep and is divided into subunits by a grid with a precise 3 mm by 3 mm surface area (Figure 2-2). The surface area of the grid used in a procedure thus determines volume of the hemocytometer containing the cells counted. One can mathematically determine the number of cells in a cubic millimeter (i.e., mm^3) (see Figure 2-2) based on this volume. One should understand these calculations so that the hemocytometer can be used to count cells in other fluids such as cerebrospinal fluid. In the United States, hematologic cell concentrations are reported in cells/µl, which equals cells/mm^3. Most other countries report hematologic cell concentrations in cells $\times$ 10^9/L. A liter contains one million (10^6) µl; therefore a liter would have a million times more cells than 1 µl (see Chapter 1).

For ease of counting, blood is first diluted with Unopette containers. The amount of dilution varies with the type of cell counted. The goal is to obtain a hemocytometer cell concentration that is neither consistently too dense to count each cell nor too dilute for accurate cell counts. For RBC counts, blood is diluted 1:200. Because blood contains fewer WBCs than RBCs, WBC dilution is usually 1:20. Platelets are diluted 1:100. The reciprocal of the dilution (i.e., 1/dilution) is used in calculation of the final conversion factor (Table 2-4).

The portion of grid in which cells are counted varies for each cell type. Because RBC numbers in blood are high, only one fifth of a square millimeter (0.2 mm^2) is counted within the center grid (see Figure 2-2). For WBC counts, the outer 4 squares are counted. For platelets, 2 squares are counted. The reciprocal of the area is used in calculating the final conversion factor. Because the hemocytometer is only 0.1 mm deep, counting 1 mm^2 represents one tenth of 1 mm^3.

To convert cell counts to number/mm^3, cell count must be adjusted for the portion of 1 mm^3 counted and dilution of the blood sample (see Table 2-4). The WBC factor of 50 is based on adjustments for depth of the hemocytometer (0.1 mm), area counted (4 mm^2), and dilution factor (1:20).

Hemocytometers have two counting chambers. Both chambers should be filled and counted. The number of cells counted from each side should vary by less than 10% for WBC counts and 20% for RBC counts. If the cell suspension was not evenly distributed, as indicated by greater variation, the test should be repeated. If the two cell counts are consistent, the average of the two sides is multiplied by the conversion factor to obtain the cell count per microliter.

Manual counts with a hemocytometer have significant error (e.g., 20% error may occur with WBC counts). Consider this magnitude of error when interpreting whether a total

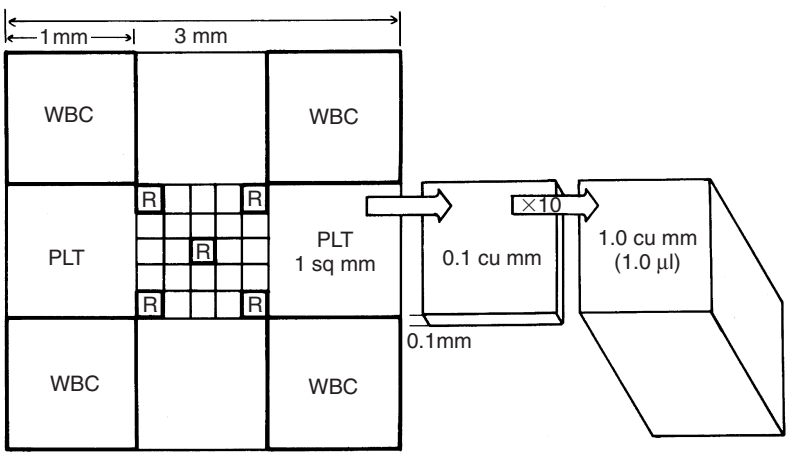

FIGURE 2-2. Dimensions of a hemocytometer. The hemocytometer grid consists of 9 mm^2. The central 1 mm^2 is additionally divided into 25 squares. The areas usually used for erythrocyte, leukocyte, and platelet counts are indicated by *R*, *WBC*, and *PLT*, respectively. Because the hemocytometer is 0.1 mm deep, the cells counted over 1 mm^2 are in the volume of 0.1 mm^3. To determine the cells in 1 mm^3 (which is 1 µl), one should multiply by 10.

TABLE 2-4. Hemocytometer Conversion Factors

	DEPTH	AREA	DILUTION	CONVERSION FACTOR
Conversion factor	10/1 x	1/area (mm^2) x	1/dilution x	
RBC factor	10/1 x	25/5 x	200/1 x	= 10,000
WBC factor	10/1 x	¼ x	20/1 x	= 50
Platelet factor	10/1 x	½ x	100/1 x	= 500

RBC, Red blood cell; *WBC*, white blood cell.

WBC count has truly changed. A count of 2,100 WBC/µl varies too little from a count of 1,900 WBC/µl the previous day to be considered an improvement.

Corrected White Blood Cell Count

Because nucleated red blood cells (NRBCs) are included in manual and automated total WBC counts, it is necessary to reduce this WBC count (actually a nucleated cell count) to account for NRBCs counted. The correction is made if more than five NRBCs are noted while counting 100 WBCs in a WBC differential count (> 5% error). The number of NRBCs seen while counting 100 WBCs (NRBC/100 WBC) is determined during the differential leukocyte count. A proportion is used to mathematically adjust the nucleated cell count as follows:

$$\text{Corrected WBC count} \div \text{nucleated cell count} = 100 \div (\text{NRBC} + 100\ \text{WBC})$$

or

$$\text{Corrected WBC count} = 100 \div (\text{NRBC} + 100) \times \text{nucleated cell count}$$

Absolute Nucleated Red Blood Cell Count

The absolute NRBC count is the difference between the nucleated cell count and the corrected WBC count. Reporting only the relative ratio of NRBC/100 WBCs can be misleading if the WBC count is very high or low. In these cases, the absolute NRBC count (per liter or microliter) is better.

Erythrocyte Indices

The RBC indices describe the average size and Hgb content of RBC. Indices may be calculated from directly determined measurements (i.e., PCV, RBC count, Hgb) by the following equations. Automated cell counters may directly measure cell volume or cell Hgb concentration.

Mean corpuscular volume (MCV) in femtoliters (fl):

$$= \text{PCV} \times 10 \div \text{RBC}\ (10^6)$$

Mean corpuscular hemoglobin (MCH) in picograms (pg):

$$= \text{Hgb} \times 10 \div \text{RBC}\ (10^6)$$

Mean corpuscular hemoglobin concentration (MCHC) in g/dl:

$$= \text{Hgb} \times 100 \div \text{PCV}$$

MCV indicates average size of the RBCs. An increased, normal, or decreased MCV indicates the average RBC was macrocytic, normocytic, or microcytic, respectively. A normal or decreased MCHC morphologically indicates the RBC was normochromic or hypochromic, respectively. Hyperchromic RBC (i.e., increased MCHC) indicate a laboratory or sample error, such as hemolysis or Heinz bodies, is present. MCH indicates Hgb content per average RBC. MCHC indicates concentration of Hgb in an average RBC. Clinically, these tests are redundant and MCH is frequently ignored.

Automated Hematology Cell Counters

Impedance Counters

The impedance principle has been and remains a standard method of cell counting in hematology instruments. Impedance counters count cell numbers, measure cell size, and mathematically calculate the Hct, MCHC, and MCH. The impedance counting principle (i.e., Coulter principle) is that cells are diluted in an electrolyte solution and drawn through an aperture. The electrical resistance across the aperture varies according to size and number of cells passing through it. The frequency of the changing resistance indicates cell numbers, and magnitude of the change indicates cell size.

One should inspect the platelet-erythrocyte histograms for adequate separation between

the platelet and erythrocyte peaks. The histograms show what cells the instrument counted as platelets or RBCs, and laboratory error can be detected (Figure 2-3). There must be clear separation between the RBC and platelet peaks for impedance instruments to count cells accurately. With small RBCs in iron deficiency anemia, large platelets, or both, the counts may be very inaccurate. The percent change in platelet count is much greater than percent change in the RBC count. This is a common laboratory error that occasionally gets out of the laboratory and to the veterinarian.

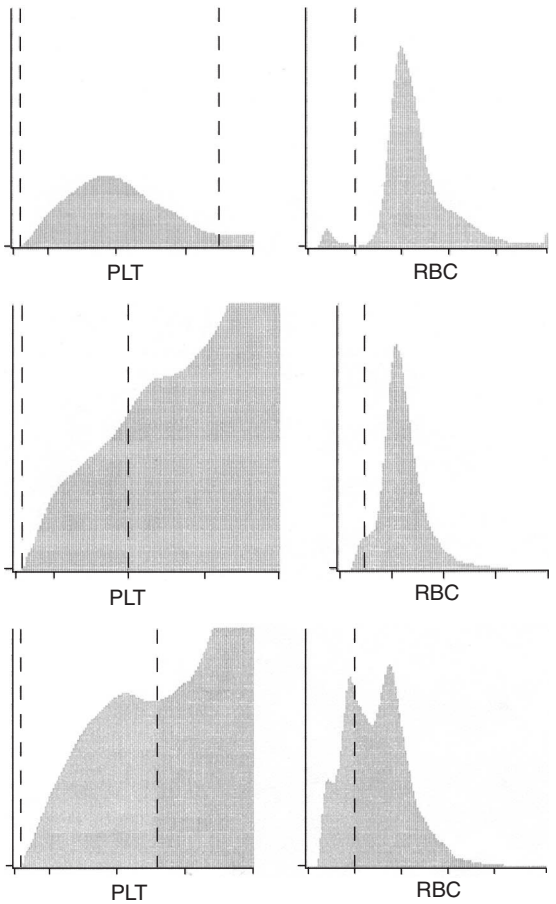

FIGURE 2-3. Three examples of red blood cell (RBC)-platelet and platelet histograms. The upper (**a**) is a normal dog. The largest peak on the right *(over letters RBC)* includes RBCs. The smaller peak left of it is the platelets in the same blood. The platelet peak is enlarged as the PLT histogram on the left *(over letters PLT)*. An impedance instrument (Cell Dyn) is used to measure the cells based on size/volume. When the platelets and RBCs are distinctly different in size, the instrument can differentiate and count them accurately. The middle (**b**) and lower (**c**) examples are iron deficiency anemia in a dog. Separation between the RBCs and platelet peaks is not clear, so the platelet and RBC counts were in error. The RBC peak in the middle example is shifted to the left and is not over the letters RBC *(or second mark on the x axis)*. The lower example was after iron treatment and there were three peaks. These were *(from left to right)* platelets, microcytic RBCs, and normocytic RBCs.

Impedance counters must be electronically adjusted to count different sized cells by electronic thresholds established for each species. Canine blood cells are similar enough to human blood cells that few problems occur. Feline blood has smaller RBCs and frequent platelet clumping, causing more concern.

In impedance counters, the Hct is calculated; it is not determined by packing RBCs by centrifugation. Therefore it is more precisely called the *Hct* instead of a *PCV*. For simplicity, PCV is usually used in this book to indicate Hct or PCV. When MCV is directly measured, PCV is calculated by the following equation:

$$PCV = MCV \times RBC \div 10$$

PCV, determined by the microhematocrit method, and the Hct, determined by an electronic cell counter, frequently vary slightly. If the variation in PCV between the two methods is greater than 3% to 5%, the technical problem may be significant enough to investigate. Hgb is determined by optical density of lysed blood, and the RBC indices are calculated.

Newer impedance instruments provide additional information, such as partial differential leukocyte count, MPV, platelet distribution width (PDW), and reticulocyte count, and they flag the presence of NRBCs. Knoll (2000) reviewed some currently available models, laser cell counters, and the QBC Vetautoread System.

Laser Cell Counters

Certain automated hematology analyzers use a laser detection system in a flow cytometer to measure size and internal complexity of cells based on light scatter at different angles. Two common veterinary instruments are the Bayer Advia 120 (Bayer H-1* is an earlier model being phased out of use) and the Abbott Cell-Dyn 3500†. The Cell-Dyn uses impedance technology for RBCs and platelets (see Figure 2-3). These provide abundant information about each cell type. The RDW describes the variability in size of the RBCs; the HDW describes variability in Hgb concentration among cells (see Chapter 3).

*Bayer Corp., Tarrytown NY.
†Abbott Cell-Dyn 3500, Abbott Diagnostics, Abbott Park, IL.

Graphic displays of cells aid in diagnosis (Tvedten, Scott, and Boon, 2000). The Bayer systems (Advia 120 and H-1) do a good job of identifying changes in RBCs caused by measuring and reporting the volume and Hgb of each RBC analyzed (Figures 2-4 and 2-5). RBC cytograms illustrate normal and abnormal RBC populations based on cell size and Hgb concentration. The Advia 120 automated reticulocyte analysis provides many newer descriptions of reticulocytes that may be useful diagnostic features after they have been validated in dogs and cats. The color-coded reticulocyte graphics show at a glance how regenerative a canine anemia was (see Figure 2-5). Advia 120 reticulocyte analysis works well for the dog but not for feline reticulocytes.

The Bayer H-1 system evaluation of WBCs includes an automated differential WBC count, which is reasonably useful for dogs (Tvedten and Haines, 1994) but more problematic for cats (Tvedten and Korcal, 1996). The various subpopulations of WBCs are displayed in a peroxidase and basophil cytogram (Figure 2-6). Patterns in the WBC cytogram are useful for detecting errors in classification of WBC types and in detecting blast cells in leukemia, NRBCs, eosinophilia, left shifts, and toxic changes in neutrophils. WBC counts are performed with the two different systems, and the instrument flags the operator (as a quality control step) when a significant difference in the WBC count or other inconsistency is detected.

The Advia 120 determines the platelet count by size and internal complexity of platelets, whereas most instruments identify platelets only by size (i.e., impedance principle). Platelets up to 60 fl are counted, which cannot be done by size because RBCs are often smaller than 60 fl (especially in cats). Figure 2-7 shows a normal canine platelet histogram and cytogram, as well as how the platelet graphics detected that a nonplatelet population (e.g., RBC fragments) was erroneously included in the platelet count. The mean platelet volume (MPV) indicates average size of platelets. The platelet cytograms

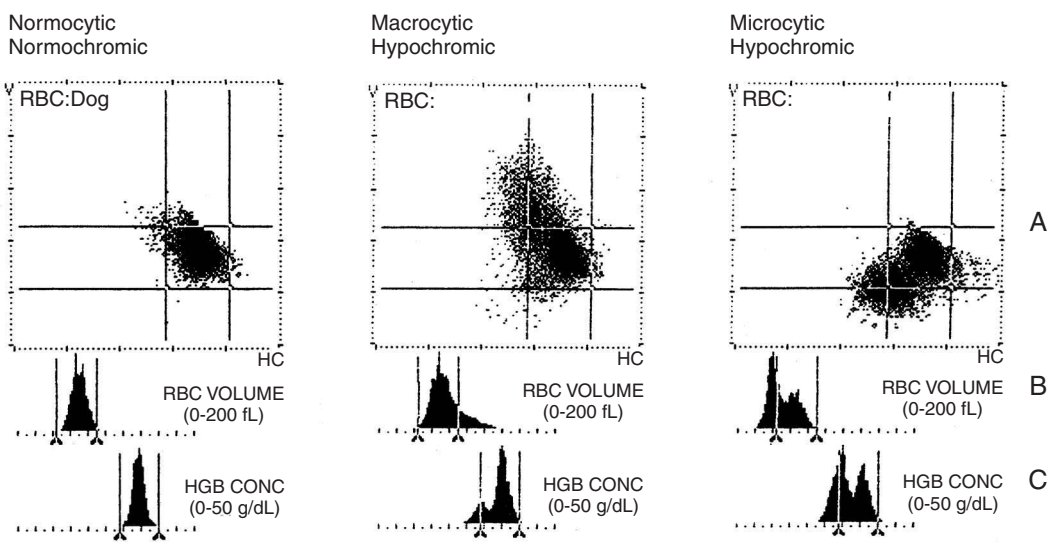

FIGURE 2-4. Erythroid portion of a Bayer H1 or Advia 120 report. The red blood cell *(RBC)* cytograms form the top row (**A**) and are a nine-box grid. The RBC cytogram is based on RBC volume *(V)* on the vertical axis and RBC Hgb concentration *(HC)* on the horizontal axis. The three RBC patterns illustrated are the three expected in most anemias. With the canine RBC cytogram on the left, the normocytic normochromic cells are in the central box. The cytogram in the center shows macrocytic hypochromic cells, mainly reticulocytes, extending toward the upper left from the normocytic normochromic cells. The cytogram on the right, from a dog with iron deficiency anemia, shows a second population of microcytic hypochromic cells adjacent to and to the lower left of the normal RBCs. The RBC volume histogram (**B**) illustrates the distribution of RBCs based on volume. The RBC histogram in the center, with immature RBCs, shows a small to medium sized tail of macrocytic cells extending to the right. The volume histogram of the dog with iron deficiency anemia on the right side has a tall peak of microcytic RBCs to the left of the normocytic RBCs. **C,** The hemoglobin (Hgb) concentration histogram *(HGB CONC)* illustrates the distribution of RBCs based on each RBC Hgb concentration. The Hgb histograms in the center and on the far right show distinct peaks of hypochromic cells to the left of the normochromic RBCs. The histograms allow estimation of the approximate magnitude of the abnormal cell population compared with normal RBCs.

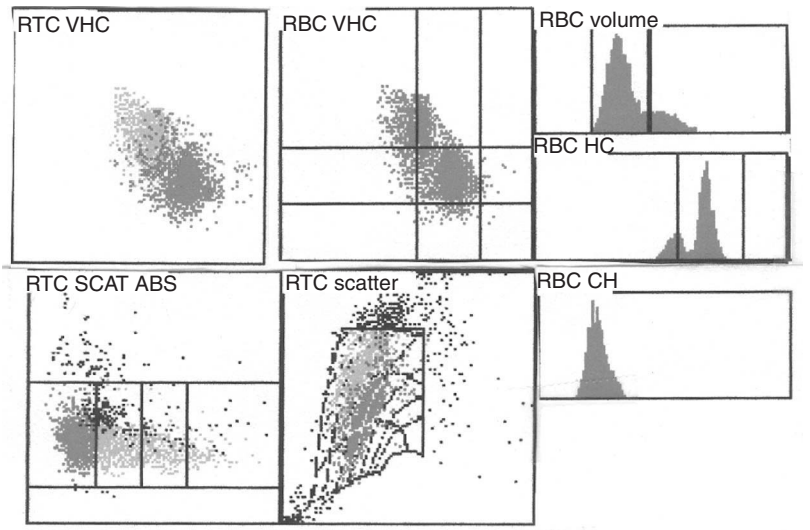

FIGURE 2-5. Red blood cell (RBC) and reticulocyte graphics from the Bayer Advia 120 showing the erythroid cells from a dog with a regenerative anemia. Two separate RBC populations on the RBC cytogram were normal RBCs and macrocytic hypochromic RBCs. The upper left cytogram *(RTC VHC)* showed the macrocytic hypochromic RBCs were the same cells that took up the reticulocyte stain and were counted as reticulocytes in the *RTC SCAT ABS* cytogram. The *RBC VOLUME* histogram *(upper right)* shows a smaller second peak of macrocytic RBCs on the right. The *RBC HC* cytogram shows a similar sized (about one third the size of the normal RBC peak) of hypochromic RBCs to the left of the larger normochromic peak. The area under the curves of the histograms reflect the relative number of normal or macrocytic-chromic RBCs, and exact numbers are available from the instrument. The histogram of cell hemoglobin (Hgb) content *(RBC CH)* failed to show the presence of two RBC populations.

and histograms illustrate distribution of platelets in the sample based on size and internal complexity. The mean platelet component concentration (MPC) is thought to detect activated platelets. Various flags and cytograms warn of potential errors (e.g., clumped platelets).

Quantitative Buffy Coat Vetautoread

The Quantitative Buffy Coat Vetautoread (QBC V) (IDEXX Inc.*) is an in-office hematology analyzer. Cell counts in the QBC V are determined from width of various layers of different cell types in an expanded buffy coat. Boundaries of the layers must be distinct enough to measure. The QBC V determines a two to three part WBC differential count consisting of mononuclear cells (i.e., lymphocytes, monocytes) and granulocytes or neutrophils and, in dogs, eosinophils.

QBC V analysis is more rapid and precise than manual counts. Based on correlation to reference methods, the correlation was excellent (r = 0.93 to 0.99) for Hct, total WBC count, and granulocyte count; good for nongranulocyte count (r = 0.81 to 0.93); and fair for platelets (r = 0.59 to 0.78) (Levine et al, 1986; Brown and Barsanti, 1988). Perfect correlation is indicated by an r value of 1.0, and greater than 0.80 is generally acceptable correlation for a new laboratory test compared with a reference method. Additional methods are also used to validate a new procedure. The precision of the QBC V, based on the coefficient of variation (CV), was better than manual methods and worse than impedance (Coulter**) counters (Brown and Barsanti, 1988). The instrument cost about $9000, and each analysis tube cost about $3.00 (Knoll and Rowell, 1996).

BLOOD SMEAR ANALYSIS

Evaluation of a blood smear by a skilled observer is a rapid source of abundant information and, therefore is an essential part of

*QBC Vetautoread Hematology System, IDEXX Inc., Westbrook, ME.

**Coulter Electronics, Inc., Edison, NJ.

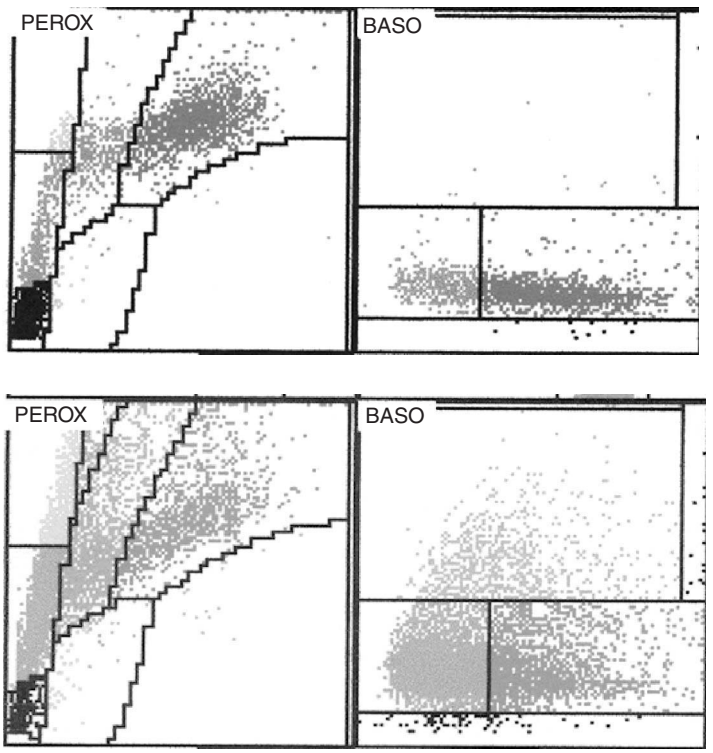

FIGURE 2-6. Peroxidase *(PEROX)* and basophil *(BASO)* cytograms from the Bayer Advia 120, showing WBCs from two dogs. The upper two cytograms are from a normal dog. The vertical (y) axis of the peroxidase cytogram is cell size, and the horizontal (x) axis is the peroxidase content of the cells. The cell types are identified by color in both cytograms. Neutrophils have abundant peroxidase, so they are located to the right. Eosinophils have much peroxidase and are found normally below the neutrophils. This example had rare eosinophils (only 4 dots). Lymphocytes lack peroxidase, are small, and are at the left. Monocytes have a little peroxidase and are located between the neutrophils and lymphocytes. Platelets, platelet clumps, and cell debris are very small and at the lower left. Large unstained cells (LUC) are at the upper left. LUC is not a cell type but a name used for cells of certain staining and size characteristics. LUC are not found in the other identifiable cell clusters. LUC include large lymphocytes, monocytes, and blast cells. The basophil cytogram has cell size on the vertical axis and nuclear density on the horizontal axis. The cytoplasm is stripped from most cells, so usually only nuclei are evaluated. The cytogram is shaped like a worm. The head of the worm contains the nuclei of mononuclear cells such as lymphocytes and monocytes. Granulocytes *(G)*, mainly neutrophils, have complex, dense lobulated nuclei, and these appear as the body of the worm. The lower cytograms were from a dog with myelomonocytic leukemia. Blast cells in large numbers are in the LUC area and upper lymphocyte area of the peroxidase cytogram and extend up from the head and back of the "worm" of the basophil cytogram. The pattern is called "bad hair day." The unique pattern allows a diagnosis of leukemia at a glance. The blast cells in the basophil cytogram extend into the basophil counting area causing an erroneous increase in the "basophil" count.

the CBC in ill animals. Automated differential cell counts from the various cell counters (described later) provide useful information but are not an alternative to blood smear evaluation in ill animals. The clinician should note that analysis of blood from individual ill animals in clinical settings differs greatly from analysis of large numbers of normal or near normal animals in research settings. Blood smear evaluation is needed to check accuracy of cell counts, produce full five- to six-cell differential leukocyte counts, and evaluate morphologic alterations in RBCs, WBCs, and platelets. None of the automated instruments consistently identify all types of cells in all species. Immature neutrophils, toxic change in neutrophils, reactive lymphocytes and monocytes, basophils, gray eosinophils, and leukemic cells typically are inconsistently identified. The automated differential leukocyte counts and cytograms and histograms are helpful and, when combined with blood smear evaluation, allow more complete and powerful conclusions. Blood smear evaluation can be used to quickly detect clinically significant changes in WBC and platelet numbers. RBC estimates are inconsistent, so the microhematocrit

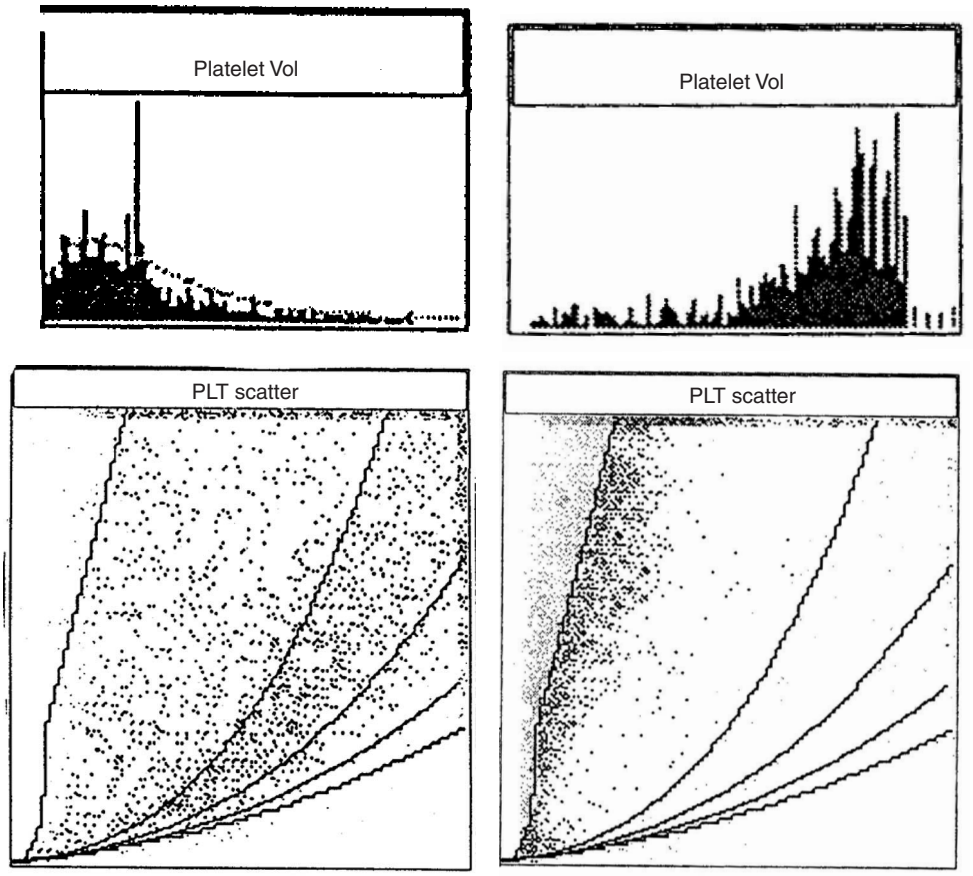

FIGURE 2-7. Advia 120 platelet histograms and cytograms from two dogs. The histogram and cytogram on the left were from a dog with iron deficiency anemia but look relatively normal. The upper-left platelet volume *(Platelet Vol)* histogram shows the number of platelets of increasing size *(left to right along x axis)*. The mean platelet volume (MPV) was about 4 to 6 fl, so the main peak of the histogram would be at about 4 to 6 fl. The histogram to the right shows a reverse tendency with mainly large cells and particles shown with a peak far to the right side of the histogram. The platelet cytograms *(PLT Scatter)* below show platelets based on size and optical density. The line that approximately follows the *y* axis up to the top indicates increasing size. The top is 30 fl, so platelets from 30 to 60 fl in size are along the top edge of the box. Most dots (i.e., platelets) are within the counting area. The platelet cytogram to the lower right was from a dog with Cushing's disease, Lipemia, and many red blood cell (RBC) ghost cells. Most of the RBC ghost cells are in a cluster along the left edge of the counting area *(line showing increasing cell size)*. Half of the ghost cells are outside of the counting area and have a different color (not considered a platelet). This was a laboratory and sample error. The instrument was confused by a lack of platelets and by the presence of a large number of cells (partially lysed RBC ghost cells) that moved into the region of size and optical density defined as platelets. The graphics allow the instrument operator to identify the error.

should be determined for quantitation of RBCs. Several qualitative morphologic observations of WBCs, RBCs, and platelets permit clinically useful conclusions. The section on cytologic smears in Chapter 16 has additional information on preparation, staining, and evaluation of smears.

Making the Smear

Smears must have a thin area where cells are distributed in a monolayer. This area provides optimal morphologic detail and reasonable cell distribution (Figure 2-8). Cells in the monolayer should be close but infrequently touching. Cells should not be distorted. WBCs must lie flat and expose a large surface area for viewing (see Figure 16-2). Cells in thin layers stain best, allowing good evaluation of cytoplasmic and nuclear detail.

The monolayer area is the only place where cell evaluation should be performed. Two common errors in reading blood and cytologic smears are (1) to try to identify cells in thick areas of the smear and (2) to try to identify damaged cells. In thick areas, cells are

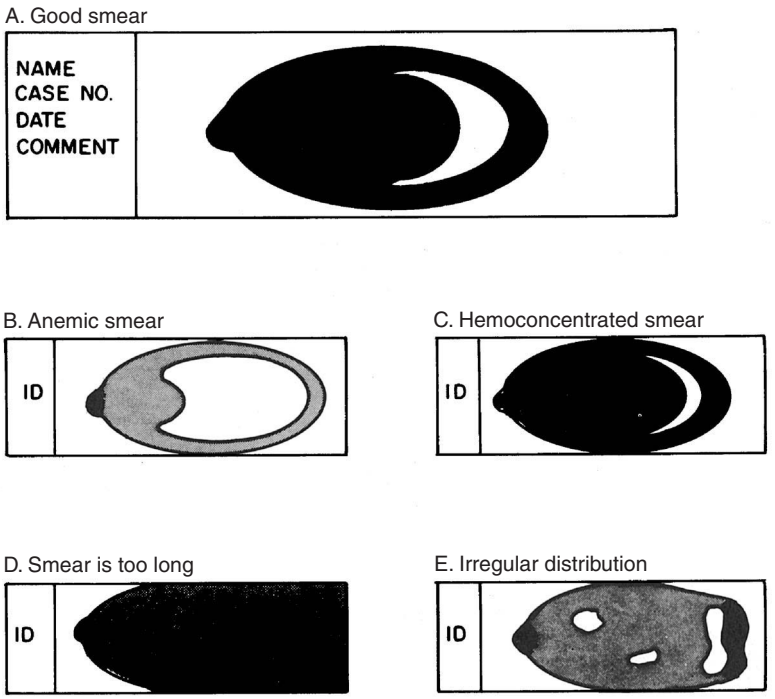

FIGURE 2-8. Examples of blood smears. A good blood smear **(A)** is properly labeled with the animal's identification, date, and other information needed to attribute any abnormality to the correct patient and sample. The smear should include an evenly distributed thin area near the end of the smear. A clear elliptic area to the right of the smear illustrates this thin monolayer. The more distal end of the smear has physical distortion and crowding of cells. Smears of anemic blood **(B)** are thin over much of the smear. The thin monolayer *(illustrated in **B** by a large clear area)* extends back toward the site where the drop was applied, as on the left end of the drawing. In smears of hemoconcentrated blood **(C),** the thin monolayer is only a narrow area near the distal end of the smear or may be absent. If too large a drop of blood is used for the smear, the smear **(D)** extends off the slide, leaving no monolayer region for cellular evaluation. Smears with uneven distribution **(E)** are caused by use of poor quality glass slides or by not making a smooth push while streaking the slide.

rounded up so that when viewed from above, the cell has a smaller diameter and stains too darkly for proper evaluation. A differential cell count cannot be accurate if unidentifiable or questionable cells are included. All smears have some damaged cells with distorted shapes and staining characteristics, which should be disregarded.

The technique of making a smear should be practiced to produce good, readable smears for personal use or to submit to a consultant. Air-dried blood smears must be made when the blood is collected to avoid cell deterioration. They should be mailed with the EDTA blood to a referral laboratory. One makes blood smears by placing a small drop of blood on one glass slide and using another spreader slide to make the smear. The spreader slide should be drawn back across the slide going through the drop of blood. Without pausing, the spreader slide is pushed forward with a single smooth stroke (stopping to permit the blood to spread along the spreader slide would result in altered WBC distribution in the smear). The angle of the spreader slide can be changed to vary the size of the smear (i.e., a lower angle produces a longer smear). A common mistake is to make a smear that extends to the end of the glass slide, causing the monolayer area to be lost (see Figure 2-8). Using too large a drop of blood to make the smear or having too low an angle on the spreader slide causes smears that are too long. The smear should only extend to the middle or distal two thirds of the slide so that the monolayer is in an area of the slide that is easily stained and examined. The size of the drop can be regulated if it is applied with a microhematocrit tube. If one stops partway through the spreading motion (i.e., no follow-through), a thick wave of blood lies where the spreader slide stopped and no monolayer is formed. To prevent an irregular smear (see Figure 2-8), use good quality

glass slides that have smooth edges and a clean surface.

Stains

Stains for hematology and cytology are discussed in Chapter 16.

Evaluating Blood Smears

The clinician should establish a routine to consistently evaluate and describe blood smears. All three cell types (i.e., WBCs, RBCs, platelets) should be evaluated for distribution, quantity, and morphologic characteristics, and the clinician should not forget to conduct a platelet evaluation.

Platelet Estimation

Platelet numbers can be estimated reasonably accurately (see Figure 5-5). Between approximately 8 and 29 platelets/100x oil immersion field is expected on normal canine blood smears. The numeric estimate should be only approximate (e.g., very low, low, normal, high, very high) rather than a specific concentration (e.g., 50,000/µl). One should check for *platelet clumping*. If platelets are clumped, they are not evenly distributed, so neither an estimate nor an actual platelet count is accurate. Platelet clumping occurs frequently in cats; thus feline platelet counts are often inaccurate. Platelet clumps are large and so tend to be found at the feathered edge of the smear. Greater error in platelet counts is expected when large or numerous clumps (or both) are found. Zelmanovic and Hetherington (1998) defined clumping as three or more aggregates of three or more platelets in 10 microscopic fields using 50x objective (i.e., 500x magnification).

Platelet Morphology

Larger-than-normal platelets may indicate increased platelet production, but finding large platelets has not been diagnostic. Noting large platelets on a blood smear and, more precisely, an increased MPV did not discriminate among different causes of thrombocytopenia in dogs, including immune-mediated thrombocytopenia, bone marrow disease, and disseminated intravascular coagulation. Large platelets and increased MPV may be present in animals with regenerative anemias or leukocytosis in the absence of thrombocytopenia, perhaps because of generalized bone marrow stimulation. Long, tubular platelets are large forms that reflect how platelets may be actively shed from tubular extensions of megakaryocytes. The discovery of irregularly shaped platelets with pseudopods is probably only the result of activation during handling. *Ehrlichia platys* morula may be visible in platelets of infected dogs but usually in numbers too low to reliably detect.

Leukocyte Estimation

One method of estimating WBCs is to scan the smear using the 10x microscope objective and subjectively estimate whether the number of WBCs is more or less than normal. One should look throughout the smear and the end of the smear, where WBCs may be unevenly distributed. Appropriate adjectives (e.g., slight, moderate, marked) are added to an estimated leukocytosis or leukopenia. Another method is to count the number of WBCs in several 10x objective fields in the monolayer area, where the platelet estimate and WBC differential are performed. In the authors' laboratory, counting between 18 and 51 WBCs/10x objective field indicated a normal WBC count for canine blood smears. Correlation of the WBCs/10x field and the actual WBC count was good, as indicated by an *r* value of 0.87 (Tvedten, Grabski, and Frome, 1988). Because accuracy deteriorates when the WBCs/10x field is greater than 60 (too numerous to count accurately), one should stop counting at 60 WBCs/10x field and not attempt to differentiate the magnitude of a leukocytosis based on the number of WBCs/10x field. This method depends on even distribution of WBCs in the smear. Uneven distribution is usually the result of WBCs being pulled to the feathered edge of the smear, causing falsely low WBC estimates.

Leukocyte Aggregation

Rare dogs and cats have strong aggregation of WBCs in EDTA blood tubes. Collection of blood in other anticoagulants (e.g., heparin, citrate) sometimes prevents or slows the aggregation and allows a more accurate total WBC count. In a cat with large WBC aggregates, the WBC count was 36,920/µl in EDTA but 64,650/µl when blood was collected in heparin and immediately analyzed. The cat's WBCs also aggregated in heparin after 15 to 30 minutes, however. The agglutination is

thought to be the result of antibodies affected by anticoagulants; the effect may be transient in that it may not be noted for several days.

Leukocyte Differential Count

WBCs are reported by absolute (cells/µl) and relative (%) WBC differential counts. The manual relative differential count is determined by identifying 100 to 200 or more WBCs on a blood smear to determine the percentage of each type of WBC. The percentage of each type (e.g., 18% eosinophils) is multiplied by the total WBC count/µl to obtain the absolute count of each WBC (e.g., 1300 eosinophils/µl). It is more consistent to evaluate the absolute counts (see Chapter 4).

Leukocyte Morphology

Left Shift • A left shift specifically indicates an inflammatory disease with rare exceptions (i.e., granulocytic leukemia, Pelger-Huët anomaly). One must be accurate and consistent in identifying immature nonsegmented neutrophils (nonsegs) to maintain the specificity of the left shift. Microscopists are often inconsistent in the determination of maturity of neutrophils and thus of presence or absence of a left shift. Just as no one criterion consistently identifies a person as mature, no one criterion consistently delineates the transition of a band into a segmented neutrophil (seg). Segs acquire various morphologic markers of maturity that distinguish them from bands, such as the following:

- Shape: Lobe formation with focal narrowing (i.e., indentation) of the nuclear margin and loss of parallel sides
- Thin, dark nucleus
- Elongated nucleus
- Coarse clumping of nuclear chromatin
- Rough nuclear margin (where chromatin clumps protrude)

When uncertain if a cell is a band or a seg, one should call it a *seg* (because segs are usually more common than bands). In sepsis or endotoxemia, neutrophils develop abnormally (i.e., asynchronous maturation), making consistent identification of various stages of maturation difficult or impossible.

The magnitude of the left shift reflects the severity of inflammation. Neutrophils younger than segs include bands, metamyelocytes, myelocytes, promyelocytes, and myeloblasts. All immature neutrophils are nonsegs. Nonsegs in blood are usually bands, because the bone marrow tends to preferentially release the most mature form of neutrophils. If the left shift includes nonsegs younger than bands, each type should be noted to reflect the severity of the left shift.

Toxic Neutrophils • Significant toxic change in neutrophils (see Color Plate 2F) indicates toxemia. Toxic change should be reported both by the number of neutrophils affected and by the severity. When toxicity is mild, or few neutrophils are affected (or both), there may be no clinical evidence of toxemia; however, when toxic changes are moderate to severe in many cells, toxemia is usually clinically apparent. The most severe toxic changes are due to bacterial toxins, often related to enteric disease (e.g., parvoviral diarrhea) or other gram-negative bacterial infections. Other toxins may cause toxic changes. The following guidelines are used in the authors' laboratory for uniformity in reporting (0% to 4% toxic neutrophils is insignificant): few, 5% to 10%; moderate, 11% to 30%; and many, greater than 30%.

Severity of toxicity is subjective (Table 2-5). Mildly toxic canine neutrophils only have Döhle bodies, and when only a few are present they may be insignificant. Döhle bodies in feline neutrophils appear frequently and in cats without signs of toxemia. More severe toxicity (e.g., many 2+ to 4+ toxic neutrophils) usually reflects clinical toxemia and an increasingly worse prognosis with increasing toxicity. These animals usually have bacterial infections (e.g., gram-negative bacterial enteritis secondary to parvovirus infection). Severely toxic neutrophils have vacuolated (i.e., foamy) and basophilic cytoplasmic characteristics (see Color Plate 2F).

Reactive Lymphocytes • Reactive lymphocytes have prominent dark-blue cytoplasm that results from increased protein synthesis

TABLE 2-5. Appearance of Toxic Neutrophils and a Simplified Grading Scheme

SEVERITY	MORPHOLOGIC CHARACTERISTICS OF TOXICITY
1+	Only Döhle bodies are present
2+ or 3+	Variable intensity of cytoplasmic basophilia, foaminess, and/or toxic granulation
4+	Cells are too toxic to differentiate from reactive lymphocytes or reactive monocytes

and increased mRNA in the cytoplasm. The nucleus may undergo blast transformation and have a convoluted shape. Individual cells may be only slightly enlarged lymphocytes or may transform into large blast cells. Occasional reactive lymphocytes are common in blood smears of both ill and healthy animals. Numerous reactive lymphocytes in ill animals suggest strong antigenic stimulation. The number of reactive lymphocytes does not reliably indicate the strength of immune reactions. Reactive lymphocytes tend to be more numerous in young animals. Blast transformed lymphoid cells from immune stimulus can erroneously mimic acute lymphoblastic leukemia (ALL).

Leukemia • Most dogs and cats with leukemia have acute leukemia. Acute leukemia is usually characterized by marked leukocytosis with many blast cells (see Chapter 4). Neoplastic cells are often hard to identify to cell type and may be simply called "blasts" or "atypical cells" unless specific staining procedures are done.

Erythrocyte Estimation

Estimating RBC numbers from a blood smear is not consistent owing to variability in the thickness of manually prepared blood smears. The clinician should observe the gross staining intensity of the slide and length of the monolayer area. Blood smears from severely anemic animals grossly appear pale against a white background, whereas blood smears from patients with a normal PCV appear red or orange. In blood smears from animals with a normal PCV, the monolayer area is a small to moderate elliptical area just behind the feathered edge of the smear (see Figure 2-8). In smears of very anemic blood, the monolayer area extends farther back, even to where the drop of blood was initially applied (see Figure 2-8). In hemoconcentrated blood (e.g., dehydration), the monolayer area is small or absent.

Erythrocyte Morphology

Many RBC morphologic changes are described with Latin and English names. Some morphologic changes are diagnostically useful, such as polychromasia (i.e., reticulocytosis), microcytic hypochromic cells, spherocytes, autoagglutination, rouleaux, Heinz bodies, blood parasites, and RBCs with distemper inclusion bodies (Figure 2-9; see Color Plates 2A to 2D). Many common RBC changes are of little clinical significance (e.g., anisocytosis, echinocytes, elliptocytes, codocytes, leptocytes) especially in low numbers. See Chapter 3.

RBC morphology should be evaluated on the thick edge of the monolayer area. On the thin edge of the monolayer area (i.e., near the feathered edge), RBCs are distorted and lose their shape. One should use appearance of RBCs and WBCs to identify the correct areas of the smear to evaluate. Canine RBCs should display normal central pallor in the proper areas of the smear.

The frequency of the morphologic change in the RBC population must be known for interpretation of its significance. Abnormal RBCs are found in low numbers on blood smears from normal animals. One should disregard rare abnormal RBCs.

Polychromasia • Polychromasia, as seen in Wright's- or Giemsa-stained (i.e., Romanowsky type) blood smears, indicates increased numbers of larger, bluer erythrocytes called *polychromatophils*. Polychromatophils are canine reticulocytes or feline aggregate reticulocytes when stained with new methylene blue (NMB) or other reticulocyte stains. NMB-stained reticulocytes have dark granules in a linear (i.e., reticular) pattern. Polychromasia indicates increased release of young, macrocytic hypochromic erythroid cells from the bone marrow in regenerative anemias (see Chapter 3). Relative guidelines to quantify reticulocytes and polychromatophils in dogs are as follows: normal, 1%; slight increase, 1% to 4%; moderate increase, 5% to 20%; and marked increase, 21% to 50%. For feline aggregate reticulocytes and polychromasia guidelines are as follows: normal, less than or equal to 0.4%; slight increase, 0.5% to 2%; moderate increase, 3% to 4%; and marked increase, greater than or equal to 5% (Perman and Schall, 1983). The amount of polychromasia should be recorded.

Spherocytes • Blood smear analysis is often diagnostic in hemolytic anemias. Several morphologic changes in RBCs are specific for certain disease conditions (see Chapter 3). Moderate-to-abundant spherocytes or autoagglutination, or both indicate IMHA. To identify spherocytes in canine blood smears, the clinician should examine the monolayer area where any normal RBCs lie flat and exhibit normal central pallor (see Figure 2-9). By comparison, spherocytes appear smaller in

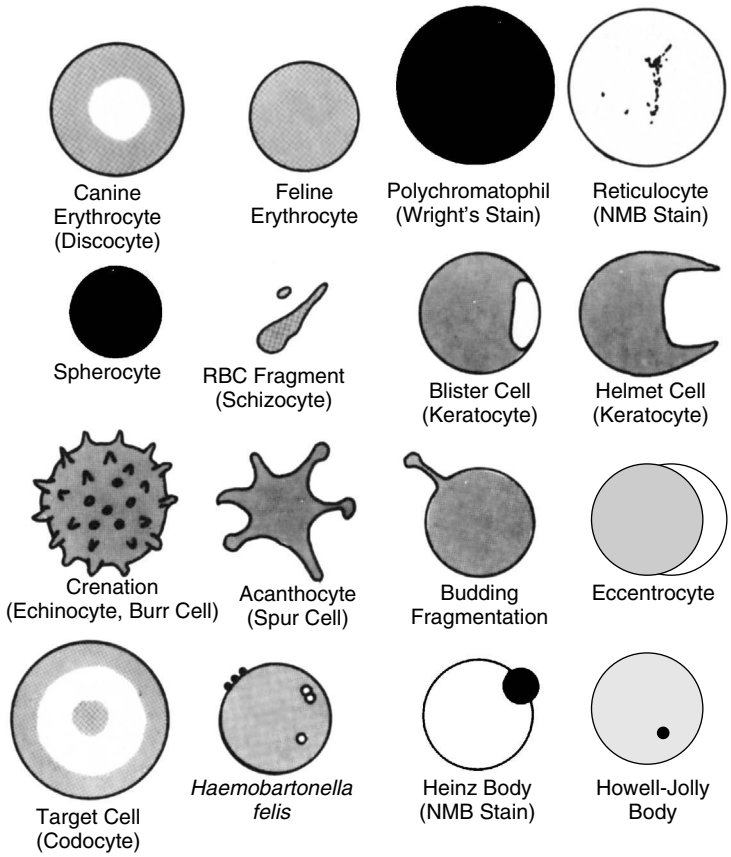

FIGURE 2-9. Selected erythrocyte terminology. Some common terms and synonyms are given beneath a drawing of selected morphologic alterations of red blood cells (RBCs). These are illustrated as they appear on Wright-stained blood smears, except for reticulocytes and Heinz bodies, which are preferentially stained with new methylene blue (NMB). Two normal erythrocytes are shown first for comparison. (See text for descriptions and diagnostic significance.)

diameter and darker orange, and they lack central pallor (see Color Plate 2C). In thick areas and near the feathered edge of blood smears, normal canine RBCs lack central pallor and mimic spherocytes, so one needs to be careful. On the thin edge of normal canine smears, RBCs are stretched out of shape and also lack central pallor. Feline RBCs normally lack central pallor; therefore evaluating the number of spherocytes is not recommended.

Autoagglutination • Autoagglutination is immune-induced aggregation of RBCs into grapelike clusters. True autoagglutination is equivalent to a positive Coombs' reaction and strong evidence for IMHA. Transfused blood may be removed by immune-mediated hemolysis, mimicking IMHA. Both autoagglutination and strong rouleaux may be visible grossly in the test tube, in which clumps of RBCs flow like sand in the waves on the beach. Rouleaux formation is the linking of RBCs into chains resembling stacks of coins. Some rouleaux is normal in dogs, and more occurs in cats. Increased rouleaux in canine blood smears usually indicates inflammatory disease. A rare exception is from antibodies produced by lymphoid neoplasia. Rouleaux is due to increased fibrinogen and gamma globulins. Autoagglutination may be differentiated from rouleaux by mixing blood with at least an equal amount of saline and observing it as a wet mount under the microscope. Saline causes RBCs to disperse from gross and microscopic clumping of RBCs caused by rouleaux but does not cause dispersal if the clumping stems from autoagglutination (see Color Plates 1B to 1F).

Other diagnostic RBC observations in regenerative anemias include *blood parasites* and *Heinz bodies*. *Hypochromic erythrocytes* and marked *poikilocytosis* occur in *iron deficiency anemia* (see Chapter 3). *Anisocytosis* (i.e., variable size) is greatest when associated with the combination of strong *polychromasia* (i.e., large RBCs) and *spherocytosis* (i.e., RBCs with small diameter) in IMHA.

Poikilocytosis • Poikilocytosis (i.e., variable RBC shapes) should be further classified

as to the type of shape change present (see Figure 2-9). Echinocytes (e.g., crenation) are usually artifacts. Echinocytes may be more numerous in some metabolic diseases. Echinocytes have numerous, uniform, usually pointed or occasionally rounded projections from the RBC surface. These projections, when viewed from above the cell, resemble dots and tiny letter *o*'s that may mimic the ring forms of *Haemobartonella*. *Canine distemper inclusion bodies* are rare in RBCs and WBCs (see Color Plates 2D and 2E) but are diagnostic when present.

Acanthocytes • Acanthocytes have a few irregular projections with rounded ends often forming a bud. Some RBCs resemble both acanthocytes and echinocytes in having sharp pointed projections that are irregular in length and few in number. They have been called *echinoacanthocytes*. In dogs, acanthocytes are associated with RBC fragmentation and with altered lipid metabolism such as occurs in hepatic disease. In cats, acanthocytes are frequently associated with liver disease, including hepatic lipidosis and cholangiohepatitis.

Red Blood Cell Fragmentation • RBC fragmentation may be the result of metabolic disorders, intravascular trauma, or iron deficiency anemia. Vascular disorders such as disseminated intravascular coagulopathy cause fibrin strands in the blood flow that can split RBCs hitting them. The small, irregular RBC fragments are called *schistocytes* (i.e., *schizocytes), keratocytes*, *helmet cells,* or *RBC fragments* (see Figure 2-9). However, acanthocytes are the most frequent shape change associated with RBC fragmentation in dogs.

Leptocytes • Leptocytes are flexible RBCs with seemingly excessive membrane. When they lie flatter than normal on the slide, one sees an enlarged area of central pallor. This wider area of central pallor of leptocytes is differentiated from the hypochromasia of iron deficiency by the thickness and color of the rim of Hgb and by RBC indices. Leptocytes have a dark rim of Hgb, whereas iron deficient cells have a thin, faint rim of Hgb (see Color Plate 2A). A common form of leptocyte is the *codocyte* (i.e., target cell), which has a small circle of Hgb in the middle of the area of central pallor (see Figure 2-9). Leptocytes and codocytes are nonspecific findings, and may be associated with regenerative anemias and splenic or hepatic disease.

Many other RBC shapes have descriptive names, such as *elliptocyte* (i.e., oval RBCs or ovalocyte) and *dacryocyte* (i.e., teardrop-shaped RBCs). If RBCs have abnormal shapes that do not easily fit the common classifications, one should simply report the presence of poikilocytosis and quantify it from 1 to 4+. Prominent poikilocytosis indicates an abnormality, but a cause may not be clear.

OTHER DETERMINATIONS

Plasma Protein Determination

Plasma total protein can be determined from the plasma layer in a centrifuged microhematocrit tube. The tube is scored with a file just above the buffy coat and broken, and the plasma is placed into a refractometer. Most refractometers have an internal scale for plasma total protein and a scale for urine specific gravity. If the scale is graduated for only refractive index, a conversion chart is necessary to convert the index to the protein concentration. Because both plasma protein and PCV are affected by hydration status, it is helpful to determine both plasma protein and PCV to evaluate anemia, polycythemia, or protein disorders.

Fibrinogen and Acute Phase Proteins

Fibrinogen is a plasma acute phase protein that begins to increase with the onset of inflammatory diseases, continues to increase for several days, and remains elevated until the inflammatory process resolves. C-reactive protein, haptoglobin, serum amyloid A and globulins in the $alpha_2$ spikes on a serum protein electrophoretogram (SEP) are other positive acute phase proteins that increase as the result of inflammatory mediators. Albumin is a negative acute phase protein that decreases in synthesis during inflammation. Fibrinogen level can be estimated by a modification of the plasma protein determination. The plasma fibrinogen level is estimated as the difference in plasma protein concentrations with or without heating plasma. One reading is a plasma protein concentration from a routinely prepared microhematocrit tube using a refractometer. The other reading is the protein concentration on plasma after heating another microhematocrit tube at 56° to 58° C for 3 minutes. Fibrinogen is precipitated by heating and then removed by centrifugation. The difference between the two readings is the fibrinogen level.

Increased fibrinogen and globulins cause increased rouleaux on canine blood smears. Rouleaux causes RBCs to sediment more rapidly. RBC sedimentation can be measured by determination of the erythrocyte sedimentation rate (ESR) to monitor inflammation. Rouleaux is prominent in cat blood; thus it is harder to note an increased rouleaux.

Lipemia, Hemolysis, and Icterus

Lipemia (turbid, white), hemolysis (red wine color), and icterus (yellow orange color) can be visually detected in plasma of the microhematocrit tube (see Color Plate 1A). Lipemia occurs with recent ingestion of a fatty meal, pancreatitis, diabetic ketoacidosis, hypothyroidism, hepatic disease, and primary lipid disorders (e.g., schnauzers; see Chapter 8). Lipemic plasma causes an altered refractive index, so plasma protein determination in a refractometer will be erroneous. RBCs are more fragile in lipemic plasma and tend to lyse in vitro, so hemolysis often accompanies lipemia. The Hgb concentration is also invalid because of increased optical density of turbid, lipemic plasma. RBCs appear fuzzy on blood smears, and the background appears blue and foamy.

Hemolysis is often an artifact of collection and handling but also is an indicator of intravascular hemolysis. Hemolytic anemia must be acute and massive to cause hemolyzed plasma, and true intravascular hemolysis should be associated with hemoglobinuria. Artifactual hemolysis will falsely decrease PCV and MCV values and increase the MCHC. Hyperchromasia (i.e., increased MCHC) is not a true increase in Hgb in RBCs, but it is due to erroneous results that may be associated with free Hgb in plasma or Heinz bodies in the RBCs (laser counters) or Heinz bodies in suspensions of lysed RBCs (Hgb determination).

Icterus suggests either hemolytic anemia or a hepatic problem (see Chapter 9). Some laboratories perform an icterus index on plasma to roughly quantify icterus. The icterus index compares the color of plasma with a set of color standards.

Color of Blood

Abnormal color of blood is evaluated by putting a drop on white filter paper. Brown blood (or perhaps only darker-than-normal blood) suggests methemoglobinemia. In cyanide poisoning, blood may be cherry red, and in carbon monoxide poisoning blood should be bright red.

BONE MARROW EXAMINATION

Bone marrow is usually examined to answer certain questions that arose from evaluating the CBC. Indications for bone marrow examination include nonregenerative anemia, persistent neutropenia, persistent thrombocytopenia, unexplained polycythemia or thrombocytosis, and atypical cells in blood, unexplained hypercalcemia, hyperproteinemia and monoclonal gammopathy, and staging of lymphosarcoma and mast cell tumors. A brief summary of general bone marrow conclusions is given in Table 2-6. For more specific details on diagnostic approaches in certain disorders of RBCs, WBCs, and platelets, see Chapters 3, 4, and 5, respectively.

Conclusions obtained from evaluation of a bone marrow aspirate and biopsy are quantitative and qualitative. The quantity of various cell types is determined. In animals with peripheral cytopenia, bone marrow examination should indicate whether decreased bone marrow production is the cause. With blood

TABLE 2-6. Basic Bone Marrow Conclusions

BONE MARROW CONCLUSION	DEFINITION
Erythroid hyperplasia	Increased proliferation of erythroid precursors
Erythroid hypoplasia	Decreased erythroid cell proliferation
Myeloid hyperplasia	Increased myeloid cell proliferation (neutrophils, eosinophils, monocytes, basophils)
Myeloid hypoplasia	Decreased myeloid cell proliferation
Megakaryocytic hyperplasia	Increased megakaryocyte proliferation
Megakaryocytic hypoplasia	Decreased megakaryocyte proliferation
Aplastic pancytopenia	Great decrease in erythroid, myeloid, and megakaryocytic cells
Lymphoid hyperplasia	Increased proliferation of lymphocytes and plasma cells
Myelodysplastic syndrome	Anemia or pancytopenia in blood, hypercellular marrow with dysplastic features
Acute leukemia	Hypercellular marrow with greater than 30% blast cells

loss (e.g., hemorrhage), blood destruction (e.g., hemolysis), or increased use of blood cells (e.g., disseminated intravascular coagulation), the numbers of marrow precursor cells should be increased (i.e., hyperplasia) after 1 to 3 days.

With decreased production, the numbers of the particular cell type in bone marrow should be decreased, although a less obvious pattern occurs with ineffective hematopoiesis. In ineffective hematopoiesis, the number of precursor cells in the marrow may be normal or increased, but they are destroyed before being released into the blood. This may be caused by infections, genetic defects (i.e., myelodysplasia, leukemia) in the cells that prevent their maturation or by autoantibodies or drugs that affect the cells.

Quantitative conclusions include megakaryocytic hyperplasia or hypoplasia, erythroid hyperplasia or hypoplasia, myeloid hyperplasia or hypoplasia, lymphoid hyperplasia, myelodysplastic syndrome, and leukemia. An adjective (i.e., mild, moderate, marked) should indicate the magnitude of the hyperplastic or hypoplastic changes.

Hyperplasia and Neoplasia

An increased proliferation of one or more cell types in the bone marrow may be a normal response to increased need for RBCs, WBCs, and platelets, or it may be an abnormal neoplastic or dysplastic process. In hemolytic or blood loss anemia, erythroid hyperplasia should develop in the bone marrow. Use of WBCs during inflammatory disease should stimulate myeloid hyperplasia. Consumption of platelets by immune-mediated thrombocytopenia or disseminated intravascular coagulopathy should provoke megakaryocytic hyperplasia. Megakaryocytic hyperplasia often accompanies regenerative anemia. A systemic immune response may result in lymphocytic plasmacytic hyperplasia. A strong shift in a cell population toward immaturity (e.g., excessive blast cells) or morphologic abnormality of marrow cells is associated with leukemia or myelodysplasia (i.e., abnormal development) (see Chapter 4).

Hypoplasia and Aplasia

The marrow may have decreased numbers (i.e., hypoplasia) or severe deficiency (i.e., aplasia) of one or more cell types. Hypoplasia or aplasia indicates that cytopenia is due to insufficient marrow production of that cell type. Specific causes of marrow hypoplasia and aplasia are described in the appropriate chapters. Peripheral pancytopenia or bicytopenia usually predict bone marrow hypoplasia.

Myelodysplastic Syndromes

Myelodysplastic syndromes identify qualitative bone marrow defects. Precursor cells in the bone marrow are normal or increased in number, but cells are destroyed before entering the blood (see Chapters 3 and 4). Myelodysplastic syndromes can be primary or secondary and are characterized by increased numbers of immature cells and morphologic alterations in precursor cells. Primary myelodysplastic syndromes have been subclassified as myelodysplastic syndromes with refractory anemia (MDS-RA), myelodysplastic syndrome with excess rubriblasts (MDS-Er), and myelodysplastic syndrome with excess myeloblasts. Secondary causes of myelodysplastic syndromes include viral, drug-induced, inflammatory, nonneoplastic, preneoplastic, and neoplastic conditions. Feline leukemia virus infection may induce dysplastic erythroid precursors including megaloblastic rubricytes with excessive amounts of cytoplasm (see discussion of vitamin B_{12}-folate deficiency in Chapter 3). The opposite nuclear: cytoplasmic (N:C) ratio occurs in iron deficiency, in which the late rubricytes and metarubricytes may have slower Hgb production compared with nuclear maturation. Other morphologic indicators of dysplasia include dwarf megakaryocytes and multinucleation.

Acute Leukemia

Acute leukemias are caused by similar genetic defects as those seen in primary myelodysplastic syndromes. The number of blast cells present in the bone marrow or blood or both primarily differentiate the disorders. Normally, blast cells account for less than 5% of the total nucleated cell population in bone marrow. In myelodysplastic syndromes, blast cells range from 5% to 30%. In leukemia, blast cells exceed 30% (see Chapter 4). Myelodysplastic syndromes may progress to acute leukemias.

Myelofibrosis and Necrosis

Myelofibrosis is bone marrow replacement by fibroblasts or collagen or reticulin fibers.

Myelofibrosis can be primary or secondary. Bone marrow aspirates from a fibrotic marrow are frequently poorly cellular and not diagnostic, so a core biopsy for histopathologic evaluation is required.

Various chemicals (e.g., chemotherapeutic drugs, estrogen) and infectious agents (e.g., gram-negative bacteria, panleukopenia virus) may cause necrosis or degeneration of the marrow. It is less frequently diagnosed than necrosis of other tissues, perhaps because the marrow is "hidden" in bone. Marrow necrosis is often diagnosed in acute toxicity studies on new drugs or chemicals at pharmaceutical or chemical companies. Scarring (i.e., myelofibrosis) may follow necrosis of marrow tissue.

BONE MARROW BIOPSY AND ASPIRATE

Best evaluation of the bone marrow's status requires all of the following: a current CBC, blood smear, bone marrow aspirate, and bone marrow core biopsy. The separate types of evidence obtained from these procedures are summarized in Table 2-7. The iliac crest is a good site for biopsy and aspirate. Cortex-to-cortex biopsies contain a certain volume of bone marrow in which to judge total bone marrow cellularity and architectural changes. Bone marrow biopsy needles (e.g., pediatric Jamshidi or Illinois) have stylets locked in the needle to prevent plugging with bone.

The bone marrow smears can be prepared from aspirated marrow or cores can be rolled on glass slides. A drop of aspirated marrow is placed on a glass slide. To prepare smear for cytologic evaluation, a spreader slide is touched to the drop then picked up and a wedge-type smear made on another clean slide (i.e., similar to the way a blood smear is made). In this way 10 to 20 slides can be made from 1 drop of marrow. Smears should be inspected for small white specks (i.e., unit particles) on the feathered edge of the smears. If not present, the slide should be tilted to allow excessive blood to flow away from bone marrow particles before making the squash preparations. A second slide is placed on the marrow, and marrow is allowed to spread between the slides. The slides are pulled apart in the same horizontal plane. Smears are stained with a Wright's type of stain, but longer staining times than blood smears are required to allow adequate stain penetration of thicker, more cellular smears.

Evaluation of Bone Marrow Aspiration Smears

A consistent pattern should be followed for describing the quantitative and qualitative aspects of the cells present in bone marrow aspiration smears. Major steps in evaluation include assessment of cellularity, megakaryocyte numbers, M:E ratio, and whether maturation in the myeloid and erythroid series is orderly. These observations can be made via the 10x scanning objective.

Cellularity

Cellularity should be evaluated as normal, increased, or decreased by observing overall cellularity and cellularity of tissue fragments present on most smears. Aspirates from a normally cellular marrow should have many more individual nucleated cells than are usually visible in blood and should include immature forms. Low cellularity may result from dilution of samples with peripheral blood. Therefore when cellularity appears low, cellularity of unit particles should be evaluated. Unit particles are present in most smears and are frequently found near the feathered edge. Within the unit particle, the percentage of space occupied by hemic cells (i.e., hematopoietic cells with dark-blue nuclei) compared with the percentage of space occupied by fat (i.e., clear, nonstaining)

TABLE 2-7. Different Information Provided by Three Tests Used in Bone Marrow Examination

TEST	INFORMATION PROVIDED
Complete blood count (CBC)	Relatively quantitative information on all three cell types in peripheral blood and excellent morphology of peripheral blood cells
Bone marrow aspirate	Excellent morphology of individual marrow cells, best determination of percentages and ratios of cell types (e.g., M:E ratio)
Bone marrow biopsy for histopathology	Best quantitation of cell numbers and iron stores in a defined volume of marrow, architectural patterns, and identification of cells that do not exfoliate (e.g., fibroblasts in myelofibrosis)

is determined. In normally cellular marrow, hemic tissue occupies 25% to 75% of the unit particle.

Megakaryocyte numbers can be readily evaluated using the 10x objective. Although normal numbers vary among samples and among slides from the same aspirate, cellular smears usually contain 10 to 60 megakaryocytes.

Myeloid:Erythroid Ratio

The M:E ratio is akin to the CBC's differential leukocyte count. To accurately evaluate cell populations, one should count 500 to 1000 cells. For clinical use, the M:E ratio may be determined by performing two or three 100-cell counts on different smears. Some experienced hematologists estimate an M:E ratio by examining smears using the 10x objective. Erythroid cells tend to be smaller than myeloid cells, have coarse nuclear chromatin, and cytoplasm that is dark blue converting to orange. Myeloid cells have nuclei with fine chromatin and gray cytoplasm with various granules and polymorphonuclear shaped nuclei in more mature cells. Normal M:E ratios for dogs are 0.75:1 to 2.5:1 and for cats are 1.2:1 to 2.2:1.

Maturation of Myeloid and Erythroid Lines

In normal marrow, 80% of nucleated erythroid cells should be relatively mature (i.e., rubricytes, metarubricytes). These cells can be identified by a densely clumped nuclear chromatin and variable hemoglobinization of the cytoplasm as identified by a gray-to-pink cytoplasm (Table 2-8). Eighty percent of myeloid cells should be metamyelocytes, bands, and segmented granulocytes. These cells can be identified by their indented to fully lobed nuclei.

Hemosiderin

Hemosiderin in macrophages appears as blue-green granules. One should report the amount of hemosiderin. Its absence in canine marrow indicates iron deficiency anemia. Excessive hemosiderin is found in bone marrow of dogs with anemia of inflammatory disease. Hemosiderin can also be excessive in dogs with persistent nonregenerative anemia given multiple transfusions or in dogs with hemolytic anemia. Hemosiderin is infrequently observed in cat bone marrow; therefore its absence cannot be used as an indication of iron deficiency.

TABLE 2-8. Approximate Percentage of Different Cell Types in Canine and Feline Bone Marrow

CELL TYPE	DOGS	CATS
Rubriblasts	0.2	0.2
Prorubricytes	3.9	1.0
Rubricytes	27	21.6*
Metarubricytes	15.3	5.6
Total erythroid	46.4	28.7
Myeloblasts	0.0	0.8
Progranulocytes	1.3	1.7
Myelocytes†	9.0	5.0
Metamyelocytes†	9.9	10.6
Bands†	14.5	14.9
Granulocytes (mainly segs)	18.7	13.5
Total myeloid	53.4	45.9
M:E ratio	1.15:1.0	1.6:1.0

*Rubricytes include basophilic and polychromic forms.
†Neutrophilic and eosinophilic forms are combined for myelocytes, metamyelocytes, and band forms.
Information modified from Jain NC: *Schalm's veterinary hematology*, ed 4, Philadelphia, 1986, Lea & Febiger.

Cellular Morphology

Cell morphology is of diagnostic significance in some cases. Dysplastic features occur in myelodysplastic syndromes, iron deficiency, some acute leukemias, and in proliferative disorders of plasma cells and macrophages.

Summary of Bone Marrow Smear Evaluation Procedure

One should do the following:

1. Quantify cell density of the smear or smears.
   a. Total nucleated cells
   b. Density of nucleated cells in unit particles
   c. Number of megakaryocytes
2. Estimate or determine M:E ratio.
3. Verify that maturation of each cell line is orderly and complete.
4. Estimate iron (i.e., hemosiderin) stores.
5. Identify morphologic abnormalities such as neoplasia, dysplasia, infections, and inflammation.
6. Summarize information into conclusions.

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3

Douglas Weiss and Harold Tvedten

Erythrocyte Disorders

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- **Determining Erythroid Regeneration**
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    - Reticulocyte Procedure
    - Automated Reticulocyte Counting
    - Absolute Reticulocyte Count
    - Reticulocyte Index and Corrected Reticulocyte Percentage
  - Canine Reticulocyte Response
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  - Polychromasia
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  - Anisocytosis, Red Blood Cell Distribution Width, Hemoglobin Distribution Width
  - Nucleated Red Blood Cells, Basophilic Stippling
  - Siderocytes, Sideroblasts
- **Anemia Classification by Mean Corpuscular Volume and Mean Corpuscular Hemoglobin Concentration**
  - Normocytic Normochromic Anemia
  - Macrocytic Hypochromic Anemia
  - Macrocytic Normochromic Anemia
  - Microcytic Hypochromic Anemia
- **Regenerative Anemia**
- **Blood Loss Anemia**
  - External Blood Loss
  - Internal Blood Loss
- **Hemolytic Anemia**
  - Immune-Mediated Anemia
    - Spherocytosis
    - Autoagglutination
    - Feline Immune-Mediated Hemolytic Anemia
    - Direct Coombs' Test
    - Cold Hemagglutinin Disease
  - Heinz Body Anemia
    - Eccentrocytes
    - Canine Heinz Body Anemia
    - Feline Heinz Body Anemia
  - Methemoglobinemia
  - Blood Parasites
    - *Haemobartonella Felis* (*Mycoplasma Haemofelis*)
    - *Haemobartonella Canis (Mycoplasma Haemocanis)*
    - *Babesia Canis*
    - *Cytauxzoon Felis*
  - Zinc Toxicity
  - Hypophosphatemia
  - Pyruvate Kinase Deficiency
  - Other Hereditary Hemolytic Anemias
- **Nonregenerative Anemia**
  - Diagnostic Approach
  - Secondary Anemia
    - Anemia of Inflammatory Diseases
    - Anemia of Chronic Renal Disease
    - Anemia of Chronic Hepatic Disease
    - Hypothyroidism and Hypoadrenocorticism
  - Severe Nonregenerative Anemia
    - Nonregenerative Immune-Mediated Hemolytic Anemia
    - Pure Red Cell Aplasia
    - Myelodysplastic Syndrome with Refractory Anemia
    - Myelodysplastic Syndrome with Erythroid Predominance
    - Congenital Myelodysplastic Syndromes
    - Iron Deficiency Anemia
  - Feline Leukemia and Feline Immunodeficiency Virus Infection
  - Pancytopenia or Bicytopenia
    - Aplastic Pancytopenia and Myelofibrosis
    - Drug-Induced Hematologic Dyscrasia

- Estrogen Toxicity
- Sulfadiazine Toxicity
- Phenylbutazone Toxicity
- Ehrlichiosis
- Feline Leukemia Virus
- Feline Immunodeficiency Virus
- *Parvovirus*
- Myelodysplastic Syndromes and Leukemias
- Macrophage Proliferative Disorders

- **Blood Transfusion and Blood Typing**
- **Polycythemia**

Relative Polycythemia

Absolute Polycythemia

- Primary Absolute Polycythemia
- Secondary Absolute Polycythemia

ANEMIA DIAGNOSIS

Anemia is the most common erythrocyte (red blood cell [RBC]) disorder. Anemia can cause various clinical signs (e.g., weakness, lethargy, exercise intolerance, heart murmur, pica) or may be subclinical and detected only during a general diagnostic workup. Likely causes of anemia are listed in Box 3-1. An approach to anemia diagnosis is outlined in Box 3-2. A detailed description follows in specific sections.

An overview of anemia classification considers the vascular system as a flexible container with input from the bone marrow and removal by a mononuclear phagocyte system primarily located in liver, spleen, and bone marrow. The three basic causes of anemia are (1) decreased RBC production by the bone marrow, (2) loss from the body (i.e., external hemorrhage), and (3) destruction in the body (i.e., hemolysis). The spleen complicates this simplistic approach, because it contains 20% to 30% of the erythroid mass and splenic contraction causes rapid changes in distribution by releasing a concentrated bolus of stored RBCs. To a lesser extent, the spleen can relax to store RBCs, removing them from circulation. Another consideration is that anemia may be caused by a combination of effects, such as both decreased RBC production and a shortened RBC life span.

The presence and severity of anemia is usually documented by packed cell volume (PCV) (see Chapter 2). Hemoglobin concentration and RBC count are equivalent indicators of the presence and severity of anemia, but for simplicity, only the PCV is used in the following discussions. An animal's hydration status must be normal before PCV properly reflects the degree of anemia. Hydration status is usually evaluated by

BOX 3-1. Common Types of Anemia

Regenerative Anemia

Blood Loss Anemia
- External blood loss
- Internal blood loss
- Iron deficiency anemia

Hemolytic Anemia
- Immune-mediated anemia
- Cold hemagglutinin disease
- Blood parasites
  - *Haemobartonella felis (Mycoplasma haemofelis)*
  - *Haemobartonella canis (Mycoplasma haemocanis)*
  - *Babesia canis*
  - *Cytauxzoon felis*
- Heinz body anemia and methemoglobinemia
- Zinc or copper toxicity
- Hypophosphatemia
- Hereditary hemolytic anemia
  - Pyruvate kinase (PK) deficiency
  - Phosphofructokinase deficiency

Nonregenerative Anemia

Secondary Anemia
- Anemia of inflammation
- Anemia of chronic renal disease
- Anemia of chronic hepatic disease
- Hypothyroidism and hypoadrenocorticism

Iron Deficiency Anemia

Bone Marrow Disorders
- Aplastic pancytopenia
- Myelofibrosis
- Pure red blood cell aplasia
- Myelodysplastic syndromes
- Leukemia
- Hemophagocytic syndromes

Drug-Induced Hematologic Dyscrasia
- Estrogen toxicity
- Sulfadiazine toxicity
- Phenylbutazone toxicity

Infections
- *Ehrlichia*
- Feline leukemia virus (FeLV)
- Feline immunodeficiency virus (FIV)
- Feline panleukopenia virus

BOX 3-2. Approach to Anemia Diagnosis

I. Determine Severity of the Anemia (See Text)
- A. Mild anemia (PCV > 30% dog, > 20% cat)
  1. Consider age, breed, and statistical chance of normality
  2. Check for laboratory or sample error; repeat venipuncture
  3. Often secondary problem, go to IV
- B. Moderate-to-severe anemia, go to II

II. Determine Bone Marrow Responsiveness
- A. No reticulocytosis or polychromasia expected during first 2-3 days or in mild anemia (PCV > 30% dog, > 20% cat)
- B. Reticulocytosis and polychromasia peak 4-5 days if bone marrow function normal
  1. Marked canine reticulocytosis > 500,000/µl
  2. Marked feline aggregate reticulocytosis > 200,000/µl
- C. Later-stage responsiveness at 7-14 days use:
  1. Feline punctate reticulocytosis, marked > 1,500,000/µl
  2. Dogs: use increase in macrocytic hypochromic RBCs
     a. RBC cytograms and histograms illustrate amount
- D. Classification by RBC indices and hematology instrument graphics
  1. Macrocytic hypochromic: regenerative anemia
  2. Normocytic normochromic: nonregenerative or preregenerative anemia
  3. Microcytic hypochromic: iron deficiency anemia
  4. Macrocytic normochromic (see text)
- E. If adequately regenerative, go to III; if inadequately regenerative, go to IV

III. Regenerative Anemia Diagnosis
- A. Blood smear analysis critical in hemolytic anemia diagnosis
  1. Spherocytes, autoagglutination, Heinz bodies, polychromasia, blood parasites, eccentrocytes, RBC fragmentation (for interpretation see text)
- B. Hemoglobinuria is best proof of intravascular hemolytic anemia
- C. Internal blood loss resembles hemolytic anemia
  1. Document hemorrhage with cytology, etc.
- D. External blood loss
  1. Often in history
  2. Tendency toward hypoproteinemia, hypoalbuminemia, or both
  3. Check for thrombocytopenia or bleeding tendency (see Chapter 5)

IV. Nonregenerative Anemia Diagnosis
- A. Way to a diagnosis varies with case presentation
- B. Use history and severity of anemia to reevaluate reticulocyte numbers to see if anemia truly nonregenerative; duration exceeding 3-4 days excludes preregenerative anemia; reticulocyte response is weak or absent 2 weeks after the cause of an anemia ceases; mild anemia will not stimulate much reticulocytosis
- C. Evaluate rest of the hematology report
  1. Microcytic hypochromic RBCs usually indicate iron deficiency anemia
     a. RBC *cytograms* and histograms more sensitive than MCV and MCHC
     b. Half of iron deficiency anemia cases regenerative
  2. Normocytic normochromic anemia most common but nonspecific
  3. Macrocytic normochromic feline RBCs without reticulocytosis, suggests FeLV-induced myelodysplasia (see text)
  4. Evidence of inflammation (see Chapter 4); anemia of inflammatory diseases is very common (i.e., mild, normocytic normochromic anemia)
  5. Evidence of leukemia or dysplastic hematopoiesis (see Chapter 4) usually indicates bone marrow evaluation; go to H
  6. Thrombocytopenia (see Chapter 5); consider *Ehrlichia* or other infections (see Chapter 15)
  7. Pancytopenia or bicytopenia indicates bone marrow disease and bone marrow evaluation; go to H
- D. Clinical chemistry profile
  1. Renal or hepatic failure causes secondary anemia (see Chapters 7 and 9)
  2. Systemic diseases have variable causes of anemia
- E. Virology, serology if infection is likely (e.g., fever, lymphadenopathy)
- F. Endocrinologic examination for hypothyroidism or other dysfunction (see Chapter 8) (e.g., mild, normocytic normochromic anemia)
- G. Toxicity
  1. Check for testicular neoplasm or access to estrogen
  2. Withhold any current drug therapy and monitor for recovery
  3. Check for toxicants in environment
- H. Bone marrow examination reveals many diagnoses (see text and Chapter 2)
  1. Myelofibrosis, aplastic pancytopenia, dyserythropoiesis, leukemia, myelodysplasia, refractory anemia with excessive blasts, etc.

PCV, Packed cell volume; *RBC*, red blood cell; *MCV*, mean corpuscular volume; *MCHC*, mean corpuscular hemoglobin concentration; *FeLV*, feline leukemia virus.

considering plasma protein (PP) concentration and PCV in combination. PP is a clinically useful, albeit crude, indicator of hydration in absence of other factors affecting PP (e.g., protein loss via hemorrhage, intestinal disease, glomerular disease). PP determination is not a sensitive test of dehydration but is easily performed and commonly available.

Severity of anemia must be considered during interpretation of complete blood count (CBC) results. Mild anemia is frequently secondary to other disease conditions

(e.g., anemia of inflammatory disease; neoplasia; and renal, hepatic, nutritional, or endocrine diseases), and these anemias resolve with correction of the primary problems, which deserve the first and most direct attention. Mild changes from reference ranges may also be because of age or breed variations, statistical chance, or sample or laboratory error.

Moderate-to-severe anemias require more active and direct diagnostic and therapeutic attention. The following PCV ranges arbitrarily classify severity of canine anemia: mild, 30% to 37%; moderate, 20% to 29%; severe, 13% to 19%; and very severe, less than 13%. In cats, arbitrary classifications are mild, 20% to 26%; moderate, 14% to 19%; severe, 10% to 13%; and very severe, less than 10%. Rapid rate of change in PCV should stimulate prompt action. Animals with chronic, severe anemia (e.g., PCVs of 5% to 8%) can exist for weeks to months without many clinical signs.

After documenting the presence of anemia and grading its severity, one should evaluate the bone marrow's erythroid production by determining the reticulocyte response (Tables 3-1 and 3-2). Blood loss or hemolysis causes regenerative anemia (see Box 3-2). A reduced or only slightly increased reticulocyte response at 3 to 5 days after onset of moderate-to-severe anemia indicates inadequate marrow production (i.e., nonregenerative anemia).

TABLE 3-1. Degree of Erythroid Regeneration in Anemia

	PERCENTAGE OF RETICULOCYTES	
DEGREE OF STIMULATION	**DOGS**	**CATS***
Normal	1	0-0.4
Slight	1-4	0.5-2
Moderate	5-20	3-4
Marked	21-50	5+

*Indicates the percentage of aggregate reticulocytes in cats. From Perman V, Schall WB: Diseases of the red blood cells. In Ettinger SJ, editor: *Textbook of veterinary internal medicine: diseases of the dog and cat*, ed 2, vol 2, Philadelphia, 1983, WB Saunders.

DETERMINING ERYTHROID REGENERATION

Reticulocyte Evaluation

Reticulocyte quantitation in blood is the most consistent way to evaluate the strength of erythropoiesis, but one must consider time after onset of the anemia (Figure 3-1) and magnitude of the reticulocytosis (see Table 3-2) during interpretation. A reticulocyte count should be performed when the PCV is less than 30% in dogs and less than 20% in cats.

Reticulocytes are immature RBCs released in increased numbers from normal bone marrow in response to anemia. Reticulocytes have ribosomes (ribonucleic acid [RNA]) for continued hemoglobin synthesis, and these appear as blue granules when stained with new methylene blue (NMB) (Figure 3-2). Feline reticulocytes are subdivided into aggregate and punctate forms. Reticulocytes are reported as absolute reticulocyte numbers per microliter of blood, reticulocyte index (RI), reticulocyte percentage, corrected reticulocyte percentage, and estimated by inspection of the number of polychromatophilic RBC on blood smears (see later). Reticulocytes are larger (i.e., macrocytes) and have a lower hemoglobin concentration than mature RBC. Therefore macrocytosis and macrocytic hypochromic anemia indirectly reflect reticulocytosis and bone marrow responsiveness (see later).

Reticulocyte Procedure

For microscopic reticulocyte determination, ethylenediaminetetraacetic acid (EDTA) blood is mixed with a vital stain (e.g., 0.5% NMB) in equal amounts and incubated for 15 to 20 minutes. This stains residual endoplasmic reticulum and appears as dark-blue granules. A smear of the mixture is air dried, and 1000

TABLE 3-2. Reticulocyte Guidelines*

DEGREE OF REGENERATION	CANINE RETICULOCYTES/μl	FELINE AGGREGATE RETICULOCYTES/μl	FELINE PUNCTATE RETICULOCYTES/μl
None	60,000	< 15,000	< 200,000
Slight	150,000	50,000	500,000
Moderate	300,000	100,000	1,000,000
Marked	> 500,000	> 200,000	1,500,000

*Used at Michigan State University and University of Minnesota as modified by Dr. D.J. Weiss.

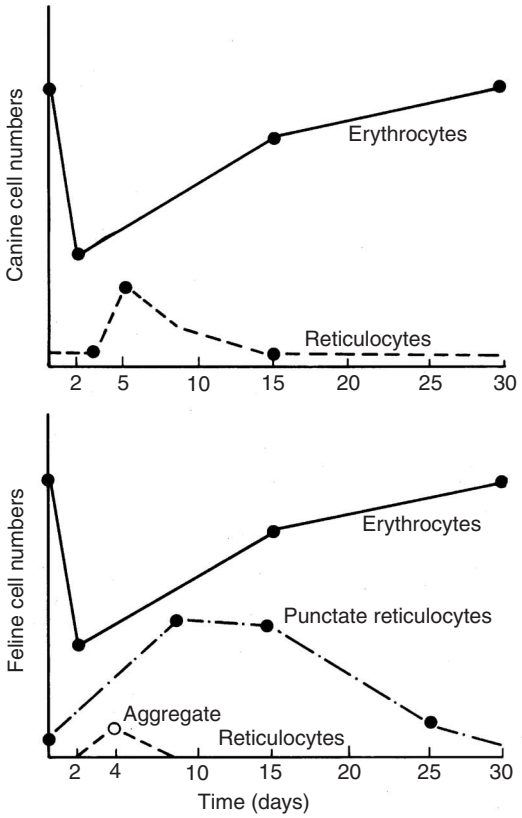

FIGURE 3-1. Reticulocyte and red blood cell (RBC) responses in blood loss anemia. The reestablishment of the erythroid mass as indicated by the packed cell volume (PCV) or RBC count is similar in both species after one episode of blood loss. It is most rapid in the first 2 weeks and is slower during the next 2 weeks. The reticulocyte responses in the dog and cat are very different. The feline aggregate reticulocyte response is weaker than in dogs but similar in onset and duration. The feline punctate reticulocyte response is uniquely long and strong.

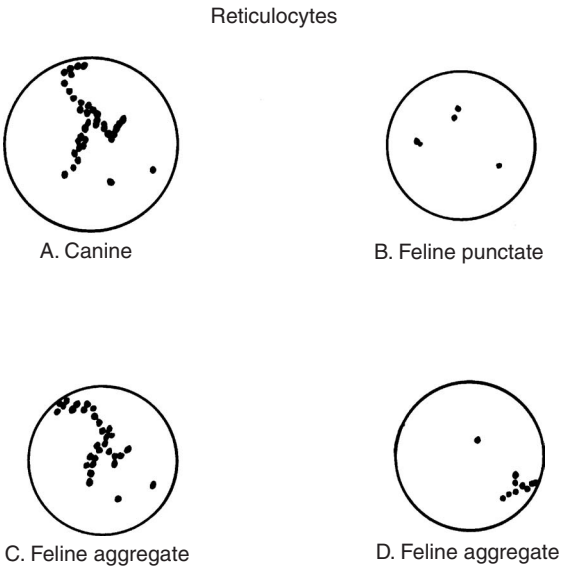

FIGURE 3-2. Reticulocytes in the dog and cat. The canine reticulocyte (**A**) usually has abundant reticulum and is considered one type. Feline reticulocytes (**B** to **D**) are subdivided into two types. The feline punctate reticulocyte (**B**) has only a few granules of RNA without aggregate formation and a 10- to 12-day life span in blood. Aggregate reticulocytes (**C** and **D**) have distinct aggregates and a half-day life span in blood.

nonnucleated RBCs are counted to determine the percentage of reticulocytes. Mature erythrocytes lack RNA and are unstained. Nucleated RBCs (NRBCs), such as metarubricytes, are younger erythroid cells also released during regenerative anemia. NRBCs are not included in the reticulocyte count but are reported separately (see later discussion). The reticulocyte percentage is imprecise, and variation among microscopists is great.

Automated Reticulocyte Counting

The Mascot Hemavet 3500* and Adiva 120 hematology analyzers determine an automated reticulocyte counts. The Hemavet uses a "focused flow" system. Erythrocytes identified as "reticulocytes" in this system are identified by size and intracellular complexity, which seems similar to the macrocytic hypochromic RBCs illustrated in Figure 2-4. These macrocytic cells usually indicate a regenerative response. The correlation of the Hemavet automated reticulocyte count with manual reticulocyte counts was only 0.67 for absolute cell counts but 0.89 for reticulocyte percentages (Perkins and Carver, 1996). The Advia 120 reticulocyte count is based on two-angle laser light scattering after staining RNA with a chromagen (see Figure 2-5).

Absolute Reticulocyte Count

The percentage of reticulocytes may be reported alone (see Table 3-1), but this can be misleading because percentage is a ratio of reticulocytes to mature RBCs. In anemia, the mature erythrocytes are variably reduced, thus the reticulocyte percentage overestimates the hematopoietic response (Figure 3-3). The absolute reticulocyte count is calculated by multiplying the reticulocyte percentage by the RBC count, so it varies with the severity of the anemia. Absolute reticulocyte count is

*Hemavet, CDC Technologies, Inc., Oxford, CT.

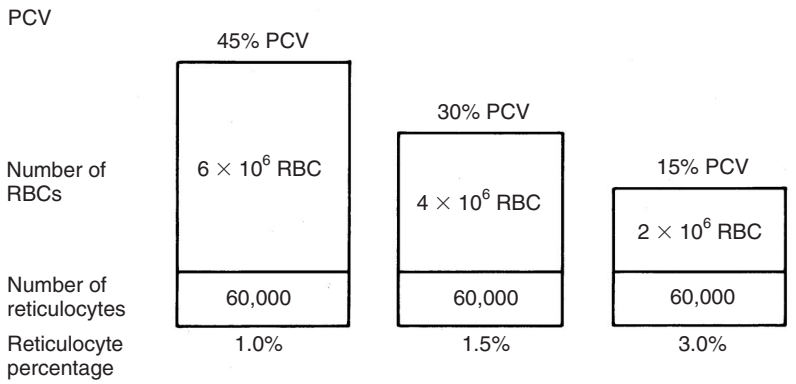

FIGURE 3-3. Increasing the severity of the anemia and thus decreasing the number of mature red blood cells (RBCs) increases the relative percentage of reticulocytes even when the absolute number of reticulocytes per volume of blood remains constant. *PCV*, Packed cell volume.

the more consistent indicator of bone marrow production and is recommended as the best single indicator of regeneration in the dog and cat. The finest gradations of regeneration are estimated with guidelines given in Table 3-2. However, absolute reticulocyte count requires the RBC count. The RBC count involves a tedious and inaccurate manual count or having access to an automated cell counter. The corrected reticulocyte percentage (CRP) and RI (also termed *reticulocyte production index*) are alternative reticulocyte production indicators that use the routinely available PCV instead of the RBC count (see later).

Reticulocyte Index and Corrected Reticulocyte Percentage

CRP is adjusted for the degree of anemia (Table 3-3). RI is further adjusted for the life span of the canine reticulocyte in peripheral blood. With increasingly severe anemia, reticulocytes are released earlier from the bone marrow and live longer in blood before maturing into erythrocytes. The longer life span increases the reticulocyte percentage, but that portion is not due to release of increased numbers of reticulocytes from the bone marrow. Feline reticulocyte life spans in blood with increasingly severe anemia are not well determined, so RI is not recommended for cats.

An RI greater than 1 in dogs indicates regenerative anemia. An RI of 3 or greater indicates a marked regenerative response. Hemolytic anemia tends to have a greater reticulocyte response than does external blood loss anemia, because the various nutrients for erythropoiesis remain in the body. Recent loss of large volumes of blood (e.g., 4 to 5 days ago) or internal blood loss, however, may also be very regenerative, so not all anemias with RI greater than 3 are hemolytic. More specific guidelines for interpreting CRP are not available.

An example is as follows: A dog with a PCV of 22.5% (see Table 3-3) had an RI of 1 that denoted an inadequate regenerative response despite an initial reticulocyte percentage of 4%. The reticulocyte percentage of 4% did not indicate a fourfold increase in reticulocyte production, though normal nonanemic dogs have less than or equal to 1% reticulocytes.

TABLE 3-3. Steps in Calculation of the Reticulocyte Index

Step 1: Corrected reticulocyte percentage (CRP)
CRP = reticulocyte % (patient's hematocrit/normal hematocrit)
Example: Dog with packed cell volume (PCV) of 22.5% and 4% reticulocytes

CRP = 4% (22.5% ÷ 45%) = 2%

Step 2: Dogs are similar to people in releasing reticulocytes from their bone marrow earlier than normal when they are anemic. These "shift" reticulocytes live longer than the usual 1 day, as is shown in the table. This exaggerates the reticulocyte percentage, so the CRP is further adjusted by dividing it by the expected maturation time in days, which varies with the severity of the anemia:

RI = CRP/life span of reticulocytes

HEMATOCRIT	EXPECTED RETICULOCYTE LIFE SPAN (DAYS)
45	1.0
35	1.5
25	2.0
15	2.5

The reticulocytes in the previous example should live about 2 days when the PCV = 22.5%. Remaining in circulation twice as long doubles the percentage of reticulocytes without the bone marrow production of reticulocytes being doubled. The reticulocyte index (RI) is adjusted for this to reflect more truly marrow release by dividing by 2.

RI = 2% ÷ 2 days = 1

The most common error in interpretation of reticulocytes is to conclude an anemia is regenerative based on slight increases in one of the ways of reporting reticulocytes.

Canine Reticulocyte Response

Canine reticulocytes normally mature to erythrocytes in about 1 day after release into the blood. As a result of this rapid maturation, reticulocytes are primarily aggregate reticulocytes (i.e., they contain a large mass of precipitated RNA with few punctate forms). Canine reticulocytes are not subdivided because the small number of punctate reticulocytes in the dog is clinically insignificant given the great variability and error potential in microscopic counts. Most laboratories do not count RBCs with only one or two individualized granules as reticulocytes. Canine reticulocytes approximately equal polychromatophils seen on Wright's-stained blood smears. Some guidelines assess the degree of activity of the bone marrow according to the percentage of reticulocytes (see Table 3-1). This was modified by Weiss to provide absolute reticulocyte number guidelines to judge the magnitude of bone marrow regeneration (see Table 3-2).

Feline Reticulocyte Response

Cats vary greatly from dogs in having very large and clinically significant numbers of punctate reticulocytes normally and in disease. A total reticulocyte count does not indicate the same interpretation as it does in the dog. Feline reticulocytes should be subdivided into punctate and aggregate to reflect significant differences in stages of maturation (see Figure 3-2). Aggregate reticulocytes mature rapidly in about half a day into punctate reticulocytes. Punctate reticulocytes mature slowly over 10 to 12 days and thus accumulate in blood in much greater numbers than do aggregate reticulocytes. The normal number of punctate reticulocyte approximates the maximum number of aggregate reticulocytes in a marked regenerative response (see Table 3-2). Reports that do not identify the type of reticulocyte counted in a cat are worthless! The duration of a regenerative anemia may be suggested by the pattern of reticulocyte response. For example, at about 4 days into a regenerative response there can be a marked aggregate response and little increase in punctate reticulocytes (see Figure 3-1). Polychromatophils on feline Wright's-stained blood smears reflect the number of aggregate reticulocytes.

In persistent hemorrhage or a hemolytic process, the combined punctate and aggregate reticulocyte count (mainly punctate) may approach 100%. This makes the performance of reticulocyte counts tedious. Even with more normal numbers of reticulocytes and no Heinz bodies, microscopic reticulocyte determinations are highly imprecise. A small Heinz body at the margin of an RBC may resemble a punctate reticulocyte, although Heinz bodies usually stain a lighter blue than do ribosomes. Flow cytometric analysis of feline blood in which the RNA of reticulocytes was stained with thiazole orange was a more sensitive and reliable assay of feline reticulocytes than manual microscopic evaluation (Perkins, Grindem, and Cullins, 1995). Reference ranges for 38 clinically normal cats by this thiazole orange method were 0.1% to 0.5% (8500 to 42,000/µl) aggregate reticulocytes and 2% to 17% (22,500 to 1,270,000/µl) punctate reticulocytes.

Polychromasia

Polychromasia denotes an increased number of polychromatophils observed on Wright's-stained blood smears. Canine polychromatophils are reticulocytes, and polychromasia indicates reticulocytosis. These are larger than mature erythrocytes and have slightly bluer staining because of ribosomes in the cytoplasm (see Color Plate 1D). Polychromatophilic refers to multiple ("poly") colors because of the orange staining of hemoglobin plus the blue staining of RNA. The feline aggregate reticulocyte appears as a polychromatophil, but the punctate reticulocytes do not. Therefore polychromasia on feline CBC reports reflects only aggregate reticulocyte numbers, whereas polychromasia on canine blood smears reflects total reticulocyte numbers. Polychromasia in both species denotes active bone marrow erythropoiesis 3 to 7 days earlier. The magnitude of the polychromasia reflects the strength of the erythropoiesis. Maximum polychromasia (4+) indicates maximum erythropoiesis.

Macrocytosis

Larger-than-normal RBCs (i.e., macrocytes) are documented by the mean corpuscular volume (MCV) or by RBC cytograms and RBC volume histograms (Figure 3-4). Reticulocytosis is the

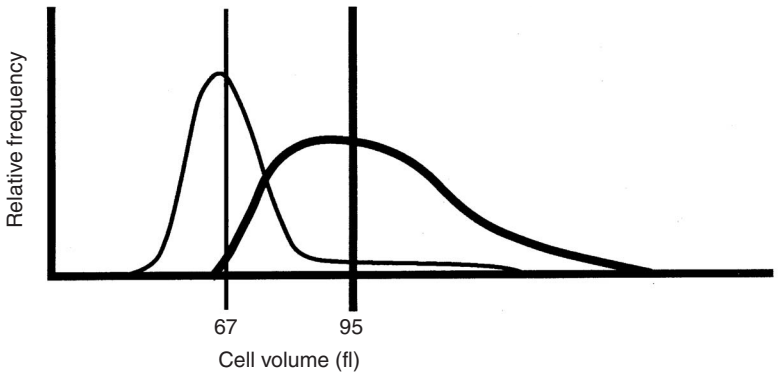

FIGURE 3-4. Example of an erythrocyte volume distribution histogram. This miniature schnauzer-beagle had stomatocytosis and secondary macrocytosis. Normal canine red blood cells (RBCs) with a mean corpuscular volume (MCV) of 67 fl are indicated *(curve to the left)*, as are the abnormal macrocytic RBCs *(curve to the right)*. There was no increase in reticulocytes. From Brown DE et al: Erythrocyte indices and volume distribution in a dog with stomatocytosis, *Vet Clin Pathol* 31:247, 1994.

primary cause of macrocytosis, especially at 4 to 5 days after the onset of anemia, but macrocytes lacking ribosomes are other RBCs released during accelerated erythropoiesis. Tvedten believes macrocytosis and especially the percentage of macrocytic hypochromic RBCs is a more sensitive indicator of increased erythropoiesis late (e.g., 7 to 14 days) in the regenerative response when polychromasia and reticulocytosis are declining (Tvedten, 1993a).

Other causes of macrocytosis are artifactual swelling of RBCs in EDTA tubes during prolonged storage, breed-associated macrocytosis (e.g., poodles), stomatocytosis (see Figure 3-4), and feline leukemia viral (FeLV) infections in cats. The macrocytosis of storage is common in samples mailed to laboratories or samples analyzed the day after collection. With the Bayer H1 RBC cytogram, this sample error can be differentiated from true regeneration because in old samples the whole, tight, single RBC cluster becomes macrocytic and somewhat hypochromic, whereas in regenerative anemia macrocytic hypochromic cells are a second population adjacent to normocytic normochromic RBCs (see Figure 2-4).

Anisocytosis, Red Blood Cell Distribution Width, Hemoglobin Distribution Width

Anisocytosis is variation in RBC size. Red blood cell distribution width (RDW) is a number derived by automated hematology counters to describe the amount of anisocytosis. A common cause of increased anisocytosis is regenerative anemia with release of immature macrocytes. Hemoglobin distribution width (HDW) numerically describes variation in RBCs based on hemoglobin concentration. Increases in RDW or HDW are indicators that the RBC population has increased variation, and one should review the blood smear for RBC morphology.

Nucleated Red Blood Cells, Basophilic Stippling

Other hematologic findings expected in regenerative anemia include Howell-Jolly bodies, NRBCs (i.e., metarubricytosis), and basophilic stippling. These are visible on blood smears but are neither as quantitative nor as specific an indicator of RBC regeneration as reticulocytes or macrocytic hypochromic RBCs.

Circulating NRBCs are reported as the number of NRBCs per 100 WBCs or NRBCs per microliter. NRBCs may be released in regenerative anemia, but NRBC numbers inconsistently reflect bone marrow erythropoiesis in dogs and cats. NRBCs release into blood may occur independently of increased erythropoiesis in splenic disease, extramedullary hematopoiesis, lead poisoning, hyperadrenocorticism, leukemia, and other bone marrow diseases.

Basophilic stippling may be seen occasionally in regenerative anemia or in canine lead poisoning. Basophilic stippling is neither a specific nor a sensitive method for diagnosing lead poisoning and when observed is not a good indicator of lead poisoning. Use toxicologic testing for lead poisoning suspects (see Chapter 17 and Appendix I).

Siderocytes, Sideroblasts

Siderocytes are abnormal RBCs with basophilic granules (i.e., Pappenheimer bodies) resembling basophilic stippling in Wright's-stained blood. Prussian blue stains the iron within Pappenheimer bodies but not granules

associated with basophilic stippling. Sideroblasts are nucleated erythroid cells in bone marrow with iron positive granules. Abnormal sideroblasts have more numerous and larger granules. Siderocytes and abnormal sideroblasts indicate abnormal erythropoiesis (i.e., dyserythropoiesis). Chloramphenicol therapy is a cause in dogs (Harvey et al, 1985).

ANEMIA CLASSIFICATION BY MEAN CORPUSCULAR VOLUME AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION

Morphologic classification of anemia traditionally uses RBC indices. MCV and the mean corpuscular hemoglobin concentration (MCHC) differentiate three common categories: (1) macrocytic hypochromic, (2) normocytic normochromic, and (3) microcytic hypochromic anemia. These categories are important in the diagnosis of anemia. The MCV and MCHC are average values of all RBCs. Therefore these values are not as sensitive an indicator of tendencies toward macrocytic hypochromic or microcytic hypochromic RBCs as are automated hematology analyzers (e.g., the Bayer Advia) (see Figure 2-4), which can identify small populations of abnormal RBCs. RBC indices often do not indicate the true anemia when only 5% to 20% of RBCs are of the altered size or hemoglobin concentration.

Normocytic Normochromic Anemia

Normocytic normochromic anemia is nonregenerative anemia with too few reticulocytes or other macrocytes to increase the MCV or decrease the MCHC. Anemia that is due to hemorrhage or hemolysis and that is of such recent onset (e.g., 1 to 2 days) as to preclude a regenerative bone marrow response is normocytic normochromic and is called *preregenerative.*

Macrocytic Hypochromic Anemia

Macrocytic hypochromic anemia is typically regenerative anemia with increased numbers of reticulocytes that are relatively larger (i.e., increased MCV) than mature RBCs. Reticulocytes are hypochromic (i.e., decreased MCHC) because they have not completed hemoglobin synthesis.

Macrocytic Normochromic Anemia

Macrocytosis usually occurs in regenerative anemia because of the release of larger, immature RBCs; the MCHC may not always drop out of the reference range, so regenerative anemia might seem like macrocytic normochromic anemia if one only uses MCV and MCHC for the evaluation. Macrocytic normochromic anemia in cats without reticulocytosis suggests FeLV infection or myelodysplasia and is not related to vitamin B_{12} or folate deficiency.

Some poodles have macrocytic RBCs (i.e., MCV > 80 fl) throughout their life but do not have anemia or show others signs of hematologic disease. Macrocytic normochromic RBCs rarely indicate a deficiency of vitamin B_{12} (e.g., malabsorption). Hypersegmentation of neutrophils and other myelodysplastic changes may also be detected in cobalamin (B_{12}) or folate deficiency. Macrocytic anemia that responded to folic acid treatment has been associated with administration of phenytoin and methotrexate (i.e., folate antagonist). A vitamin B_{12} malabsorption has been described in giant schnauzers. See also stomatocytosis discussed under Other Hereditary Hemolytic Anemias.

Microcytic Hypochromic Anemia

Microcytic hypochromic anemia is usually diagnostic for iron deficiency, which prevents adequate production of hemoglobin. The RBCs are small (i.e., low MCV) and insufficiently hemoglobinated (i.e., low MCHC). Microcytosis and altered iron metabolism is common in dogs with portosystemic shunts (PSSs) and hepatic atrophy. Note that the Japanese Akita and Shiba breeds normally have small RBCs (i.e., MCV of about 60 fl).

REGENERATIVE ANEMIA

Adults with anemia caused by RBC destruction (i.e., hemolysis) or blood loss should have normal bone marrow able to respond well to the anemia. See Box 3-1 for a listing of types of regenerative anemia. Greater than 500,000 reticulocytes/µl frequently occur in dogs with hemolytic anemia, internal hemorrhage, or recent external blood loss (see Tables 3-1 and 3-2). Many anemias, however, are neither markedly regenerative nor completely nonregenerative. Mild anemia (e.g., canine

PCV 30% to 35%; feline PCV 20% to 26%) may not stimulate reticulocytosis because the mildly stimulated bone marrow would respond by releasing mature RBCs.

Mild-to-moderate regenerative states (see Box 3-2) must be interpreted in terms of duration of anemia, severity of anemia, and potential for multiple causes. An intestinal neoplasm is an example of a situation with multiple causative factors contributing to anemia. External blood loss may result from hemorrhage into the intestine. Initially the anemia should stimulate good bone marrow regeneration, but persistent bleeding may lead to iron and protein deficiency. Neoplasms are often inflamed, and anemia of inflammatory disease interferes with erythropoiesis, resulting in reduced RBC production. Thus the degree of regeneration could vary considerably and be less than expected.

BLOOD LOSS ANEMIA

External blood loss is frequently obvious from the history or physical examination. However, external blood loss into the gastrointestinal tract or internal blood loss into a body cavity may be occult. Gastrointestinal bleeding is indicated by a black, tarry stool or fresh red blood in the stool. Tests for occult blood in the stool are of questionable value (see Chapter 9), because myoglobin present in meat diets frequently gives false positive results. Fluid cytology can document bleeding into body cavities (see Chapter 10). PCV and volume of the fluid indicate the amount of hemorrhage.

Hematologic patterns in blood loss vary greatly according to when hemorrhage occurred, severity of blood loss, whether bleeding was one acute episode or persistent, whether hemorrhage was into a body cavity or external, and species variation. Time-related changes and species variations are illustrated in Figure 3-1 and in the following discussions.

External Blood Loss

PCV in an adult dog does not fully reflect severity of acute blood loss anemia for 1 to 3 days, until fluid volume of the vascular space is replaced and the remaining RBCs and PPs are diluted. Splenic contraction in the first few hours releases stored, concentrated RBCs into the circulation and may mask the severity of anemia. Release of reticulocytes should be noticeable by 3 days after hemorrhage, and peak aggregate reticulocytosis occurs 4 to 5 days after hemorrhage (see Figure 3-1). Improvement in the PCV occurs rapidly over the first 2 weeks until PCV reaches the low-normal reference range. Thereafter, hypoxia is too mild to stimulate strong erythropoietin production, so PCV increases slowly and may take a month to return to the original PCV.

In adults, chronic hemorrhage over several weeks causes iron deficiency (see Color Plate 2A) and a negative protein balance, impairing erythropoiesis and causing increasingly weaker regenerative responses. Thus blood loss anemia initially (e.g., in the first 1 to 2 days) appears preregenerative, becomes regenerative, and then over time becomes poorly regenerative or nonregenerative because of iron deficiency. Puppies and kittens are born with small iron reserves and with maximum erythropoiesis to match growth rate; iron depletion occurs more rapidly with blood loss (e.g., hookworms or coccidia).

A low or low-normal PP level in regenerative anemia is frequently associated with external blood loss and is a useful diagnostic feature. PP is lost with the blood. In hemolytic anemia and internal blood loss, PP concentration tends to be normal to slightly increased, because no protein is lost from the body. The PP reference range may be too wide for the PP concentration to seem abnormal, but deviation from the mean PP of about 6.5 to 7.0 g/dl may indicate the trend. However, exceptions occur; for example, PP is replaced by the liver and lymphoid tissues more quickly than the bone marrow can replace RBCs, so hypoproteinemia less consistently reflects the presence or severity of external hemorrhage than does PCV.

Feline PCV response to blood loss somewhat parallels the canine response for the first 2 weeks. Feline aggregate reticulocyte response reaches a peak about 4 days after hemorrhage (see Figure 3-1) (Perman and Schall, 1983). The maximum number of feline aggregate reticulocytes is much lower than the maximum number of canine reticulocytes in strong regenerative responses. Similarly, polychromatophil numbers in strong regenerative anemia are much lower on a feline blood smear than on a canine smear. Punctate reticulocytes peak about 1 week after hemorrhage and may remain elevated for 3 weeks or more. Punctate reticulocytes may remain increased after PCV has returned to the normal range. Therefore a punctate reticulocyte

response may not reflect active bone marrow erythropoiesis.

Feline aggregate and punctate reticulocyte numbers can help determine when an anemia began. Moderately to markedly increased aggregate reticulocytes with few punctate reticulocytes indicates recent anemia (e.g., 2 to 4 days). Increased punctate reticulocytes without increased aggregate reticulocytes indicate anemia of 1 to 3 weeks' duration or anemia too mild to stimulate an aggregate reticulocyte response.

Age-related changes in puppies and kittens must be considered. The PCV in healthy 2- to 6-week-old puppies is approximately 28%, and the PP normally may be less than 6 g/dl. Puppies have approximately 3% to 7% reticulocytes at 2 months of age or younger, with the highest percentage (7%) occurring at 0 to 2 weeks of age. Conversely, adult dogs normally have less than 1% reticulocytes. Using adult reference values, one would incorrectly conclude that a puppy has anemia, reticulocytosis, and hypoproteinemia, which signify external blood loss. Therefore age-related reference values (Jain, 1986) should be used. Kittens at weaning frequently have subclinical iron deficiency that limits erythropoiesis (see the discussion of iron deficiency anemia).

Young animals are less able to respond to blood loss than are adults. Puppies and kittens already have high rates of bone marrow erythropoietic activity because of the need to expand blood volume as they grow. When bone marrow erythropoiesis is already at a high rate, one cannot expect as great an increase as occurs in adults.

Internal Blood Loss

The hematologic pattern of internal blood loss is similar to that associated with hemolytic anemia, because RBCs lost into tissues are destroyed and their constituents conserved to allow maximal bone marrow response. Some RBCs lost into the body cavities may be recirculated intact via the lymphatics.

HEMOLYTIC ANEMIA

The clinician usually diagnoses hemolytic anemia by the finding of markedly regenerative anemia without hypoproteinemia or other evidence of blood loss. Careful evaluation of the blood smear is essential to identify specific causes of hemolytic anemias, such as blood parasites, Heinz bodies, or an immune-mediated hemolytic process.

Erythrophagocytosis seen in blood smears or cytologic smears of bone marrow, spleen, liver, or lymph nodes is infrequent but a strong indication of hemolysis.

Hemolytic diseases can be subclassified as intravascular or extravascular (see Color Plates 1A and 1B). *Extravascular hemolysis* results from phagocytosis by macrophages in the spleen, liver, and bone marrow and occurs much more frequently than intravascular hemolysis. *Splenomegaly and hepatomegaly* are evidence of extravascular hemolysis. *Intravascular hemolysis* indicates an acute, severe anemia. *Hemoglobinuria* with hemoglobinemia and lysed RBCs (i.e., ghost cells) in blood smears is proof of intravascular hemolytic anemia (see Chapter 7). When intravascular hemolysis is present, extravascular hemolysis is also occurring, but one classifies the hemolysis on the most severe pattern. Heinz body anemias and complement-fixing immune-mediated anemias are the most common causes of intravascular hemolysis. Other causes are described later.

Icterus (see Color Plates 1A and 1B; see Chapter 9) occurs with intravascular and extravascular hemolysis. Increased unconjugated bilirubin production from RBC destruction temporarily exceeds the capacity of the liver to remove it. In severe anemia, the liver may also have hypoxic or toxic damage that causes decreased bilirubin metabolism and cholestasis. Bilirubin concentrations are frequently greater in intravascular hemolysis than in extravascular hemolysis because of the more rapid rate of hemolysis. Serum bilirubin determinations (i.e., total, conjugated) are of limited use in differentiating anemias from hepatic disease because many exceptions to the expected patterns occur. Hemolytic icterus classically has mainly unconjugated bilirubin, but conjugated bilirubin is also increased.

Immune-Mediated Anemia

Immune-mediated hemolytic anemia (IMHA) occurs frequently in dogs but relatively infrequently in cats. The term *autoimmune hemolytic anemia* should be avoided because "autoimmune" indicates that the immune reaction is to the animal's own antigens. *Haemobartonella*, FeLV, or drugs may induce anemia that is not autoimmune but directly or indirectly the result of a foreign antigen.

Propylthiouracil in cats and perhaps levamisole in dogs can induce IMHA. The removal of incompatible transfused RBCs is an immune-mediated hemolytic process but not an autoimmune process.

Diagnosis of IMHA is typically based on identifying large numbers of spherocytes, autoagglutination, or both in a dog that has not recently received a transfusion. The CBC pattern in canine IMHA is usually consistent and diagnostic: moderate-to-severe anemia (e.g., PCV = 16%), marked reticulocytosis (e.g., 625,000/µl), polychromasia (e.g., 3+ or 4+), normal to slightly increased PP (e.g., 7.2 g/dl), marked leukocytosis (e.g., 54,000/µl), significant spherocytosis (e.g., 2+ or more) in most (i.e., 82%) dogs, and autoagglutination and thrombocytopenia (e.g., 29%) in some dogs. The RI is usually greater than 3, suggesting hemolytic anemia. The mean RI in one study of IMHA was 4.8. Moderate-to-marked spherocytosis (e.g., spherocytes > 50% of RBCs) is diagnostic for IMHA with rare exceptions. Autoagglutination, persisting after saline dilution, is also diagnostic of IMHA. Thrombocytopenia, with concurrent with IMHA, may be immune-mediated or caused by disseminated intravascular coagulation. Some IMHA cases are nonregenerative if the immune reaction damages erythroid precursor cells in the bone marrow. A positive Coombs' (i.e., direct antiglobulin) reaction is also good evidence for IMHA.

Spherocytosis

Spherocytosis (i.e., increased spherocytes) is a subjective observation made from blood smears that is the most common, and important diagnostic feature of canine IMHA. Errors in identifying spherocytes are common, especially when evaluating RBC morphology in poor smears or improper areas of blood smears. However, spherocytes can be consistently identified in canine blood by experienced observers. Feline RBCs lack central pallor, so the presence of small RBCs in prominent anisocytosis is difficult to distinguish from spherocytosis. Spherocytes are rarely identified with confidence in cats and should not be used as a primary diagnostic feature of feline IMHA.

Spherocyte numbers affect diagnosis. Normal blood may have occasional spherocytic RBCs. A moderate-to-large number of spherocytes is required to diagnose IMHA (see Color Plate 2C). Spherocytes can be quantified in the monolayer area of a blood smear using the 100x oil objective (1000x magnification). A scale of 1+ to 4+ may be used; 1+ equals 5 to 10 spherocytes per 100x oil field (2% to 4%); 2+ equals 11 to 50 (4% to 20%); 3+ equals 51 to 150 (20% to 60%); and 4+ equals more than 150 spherocytes per field (> 60%). Note that less than 2% spherocytes is not reported.

Spherocytosis has been reported in a puppy with zinc toxicity. Zinc toxicity can cause Heinz body anemia with pyknocytes. The spherocytic portion of eccentrocytes has been misidentified as spherocytes in vitamin K_3 and zinc toxicity of dogs but these cells should be called *pyknocytes* instead of *spherocytes*. Certain snake venom intoxication may cause spherocytosis or spheroechinocytosis.

Autoagglutination

Autoagglutination, in nontransfused animals, is diagnostic for IMHA. Spontaneous autoagglutination of a blood sample is equivalent to a positive Coombs' test result because the end point of the Coombs' test is agglutination of the RBCs. Antibodies causing RBC agglutination without need for addition of Coombs' reagent are called *complete antibodies* and are usually immunoglobulin M (IgM). Autoagglutination must be differentiated from prominent rouleaux resulting from inflammatory diseases and even paraproteinemia of lymphoid neoplasia. Autoagglutination on a wet mount preparation should resemble grapelike clusters of RBCs. Rouleaux microscopically resembles linear stacks of coins and should disperse when blood is mixed with an equal or larger amount of saline in a wet mount. A drop of blood is mixed with saline on a glass slide, coverslipped, and evaluated grossly and microscopically (see Color Plates 1B to 1F). Note that the equivalent of this saline dilution occurs in hematology analyzers.

Electrolyte diluent for the analyzer disperses RBC clumping caused by rouleaux, but autoagglutination persists. Autoagglutination can be identified on RBC cytograms and causes a variety of laboratory errors in the CBC.

Feline Immune-Mediated Hemolytic Anemia

Feline IMHA is harder to diagnose than canine IHA. Spherocytosis is more difficult to identify in cats, and marked leukocytosis does not occur. *H. felis* infection induces a

Coombs'-positive IMHA (see Color Plates 1D to 1F). *H. felis* is difficult to differentiate from idiopathic IMHA if few or no organisms are found in blood smears. Recent availability of specific polymerase chain reaction (PCR) tests for *H. felis (Mycoplasma haemofelia)* has facilitated diagnosis. FeLV infection or *H. felis* infection in cats with IMHA may inhibit erythropoiesis, producing nonregenerative anemias.

Direct Coombs' Test

The direct Coombs' test identifies antibodies or complement on RBCs. The antibodies and complement may or may not be directed toward the RBC itself and may or may not damage the RBC. The usual test is a "polyvalent direct antiglobulin test" in which a polyvalent Coombs' reagent is mixed with the patient's RBCs. This reagent contains species-specific antibodies against various classes of antibodies and complement. If the patient's RBCs have enough antibody or complement to be detected and the ratio of these antibodies and the antiglobulin is proper, gross or microscopic hemagglutination occurs (i.e., a positive reaction). Some laboratories use monovalent antiglobulin to classify IMHA as either IgM or IgG types. The pattern found in 48 dogs at the University of Minnesota was that 71% were IgG positive, 10% were positive for both IgG and IgM, and 19% were positive for IgG, IgM, and complement. IgM can bind complement to RBCs and cause intravascular hemolysis and a more severe acute anemia. The effect of steroid treatment in vivo is unpredictable. Dogs remain Coombs' positive for variable lengths of time during corticosteroid treatment of IMHA.

The direct Coombs' test is neither highly specific nor sensitive for IMHA. Its result is positive in only 60% to 70% of canine IMHA cases. Possible reasons for false-negative results include insufficient quantity of antibody on RBCs, temperature at which the test was performed, improper antigen:antibody ratio, or other technical problems. To ensure that the proper antigen:antibody ratio is achieved, serial dilutions of the antiglobulin reagent should be routinely used. Over time, antibody and complement elute off the RBCs in blood samples. The time one may store an EDTA blood sample before a Coombs' test is unknown. Therefore results obtained from samples sent to laboratories by mail are questionable. Alsever's solution is good for storing antibody-coated RBCs but is not readily available to most veterinarians.

The specificity of the Coombs' test for IMHA is often considered good, but positive reactions frequently occur in the absence of evidence of IMHA. Of Coombs'-positive anemias in 134 dogs, half were positive for the third component of complement (C3), but there was infrequent evidence of intravascular or extravascular hemolytic anemia in these patients (Slappendale, 1979). Therefore a positive Coombs' test result in the absence of anemia or spherocytosis may not be caused by IMHA and can be secondary to strong immune reactions to other antigens (e.g., *Demodex,* vaccines, bee stings). Positive reactions in such conditions as RBC parasite infections, blood transfusions, and drug reactions are true reactions when they indicate immune-mediated damage to RBCs. These conditions should be classified as secondary IMHA.

Cold Hemagglutinin Disease

Rare animals have clinical disease associated with antibodies that preferentially bind to RBCs at body temperatures below normal (i.e., in peripheral capillary beds: ears or paws). If these antibodies agglutinate RBCs and occlude capillaries, poor blood flow and ischemic necrosis ensue. Cold agglutinin disease may or may not be associated with hemolytic anemia and is rarely proven in dogs and cats. The antibody expected in cold hemagglutinin disease is IgM. Not all IgM antibodies are cold antibodies, because not all IgM antibodies preferentially act at cold temperatures.

Test results for cold hemagglutinin disease should be interpreted cautiously. The disease occurs rarely enough that most laboratories are inexperienced with proper evaluation of cold agglutinins. More is involved than just refrigerating the blood sample and performing agglutination tests at cool temperatures (4° C). RBCs must be separated from the EDTA plasma strictly at 37° C to avoid binding of cold agglutinins to the RBCs. Cold agglutinins are normally found in dogs and people. In humans the titer should be greater than 1:64 to be abnormal. A diagnostic value in dogs and cats is not available. One cat with cold hemagglutinin disease had a titer of 1:52,000 (Schrader and Hurvitz, 1983). RBC agglutination occurring at 4° C should disperse when blood is warmed to body temperature.

Heinz Body Anemia

Many substances oxidize hemoglobin, causing it to precipitate and form Heinz bodies, methemoglobin, or both. Heinz bodies do not stain differentially from normal hemoglobin on Wright's-stained blood smears, but they may appear as lighter-colored, single round bodies within RBCs or may bulge from the cell surface (see Color Plate 2B). Heinz bodies stain prominently on smears stained with NMB or brilliant cresol green via the same method used for reticulocyte smears (see Figure 2-9). These substances may also form eccentrocytes.

Eccentrocytes

Eccentrocytes, like Heniz bodies, result form oxidative injury to RBC. Onion toxicity is a common cause of eccentrocytes in dogs. Onion-eating dogs may have eccentrocytes and have symptoms ranging from what appears to be no evidence of anemia up to life-threatening anemia. Eccentrocytes are RBCs in which the main cell body is more rounded (resembling a spherocyte) with a thin, eccentric lip formed of two fused layers of membrane extending along one side (see Figure 2-9). The lip of membrane contains no hemoglobin (therefore appears clear) and is often overlooked. Pyknocytes are RBCs formed by the same process but in which no lip of membrane is seen. These are often misidentified as spherocytes, which leads to an incorrect diagnosis.

Canine Heinz Body Anemia

Heinz bodies are not normally found in canine blood; therefore finding any Heinz bodies is diagnostic. Canine Heinz bodies are often small, irregular, and multiple and not always the classic, single, round body seen protruding from the surface of feline RBCs (see Color Plate 2B). Thus canine Heinz bodies may be overlooked and on Reticulocyte-stained smears may be misidentified as reticulocytes. Heinz bodies stain a lighter blue color and vary in size; therefore they can be easily separated from true reticulocytes with reticulocyte stains. Causes of canine Heinz body anemia include onions, vitamin K_3, naphthalene, propylene glycol, benzocaine, methylene blue, copper, zinc, phenylhydrazine, and acetaminophen. Ingestion of cooked, uncooked, or dehydrated onions should be the first thing ruled out when one is considering a diagnosis of canine Heinz body anemia. Vitamin K_3 is more toxic (and less effective) than K_1; therefore vitamin K_3 should not be used in treatment. S-diphenylcarbazone was erroneously used as an antidote for thallium toxicity in a dog and caused severe oxidative hemolysis.

Feline Heinz Body Anemia

Both healthy and ill cats frequently have Heinz bodies. Feline hemoglobin is more susceptible to oxidative damage, and the feline spleen is inefficient in removing Heinz bodies. Apparently healthy cats have Heinz bodies in up to 10% of their RBCs. Heinz body numbers are affected by diet (increased in cats eating fish diets and diets containing propylene glycol). Heinz bodies within RBCs are usually large (i.e., 0.5 to 3 μm in diameter) and singular. One must consider number and mass of Heinz bodies, PCV, reticulocyte response, and likelihood of exposure to oxidative toxins to determine their significance. The number of Heinz bodies in feline blood may be categorized as occasional if less than 10%, moderate if 10% to 50%, and marked if greater than 50%.

Oxidative toxins reported to cause Heinz body formation in cats include methylene blue, acetaminophen, phenacetin, phenazopyridine, propofol, propylene glycol, salmon-based diets, canned meat-based baby foods containing onion powder, and diseases including diabetes mellitus, hyperthyroidism, renal failure, and lymphosarcoma. Increased Heinz bodies in cats with diabetes mellitus, hyperthyroidism, and lymphosarcoma appear to result from altered metabolism generating oxidative metabolic intermediates (Christopher, 1989). Increased Heinz bodies in cats eating semimoist cat food diets were the result of propylene glycol (the Food and Drug Administration prohibited addition of propylene glycol to cat food in 1994).

Methemoglobinemia

Oxyhemoglobin is normal, oxygen-carrying hemoglobin. Methemoglobin is a nonfunctional form of hemoglobin formed by oxidation of ferrous iron in oxyhemoglobin to ferric iron. Methemoglobin is nonfunctional because it cannot bind oxygen. In healthy animals, approximately 1% of oxyhemoglobin is converted to methemoglobin daily. Methemoglobin reductase reduces

methemoglobin back to oxyhemoglobin. Rarely, methemoglobin reductase deficiency occurs as a congenital defect in dogs and produces significant methemoglobinemia and cyanosis. The oxyhemoglobin method for hemoglobin concentration measures only oxyhemoglobin, whereas the usual cyanmethemoglobin method measures all types of hemoglobin, including methemoglobin.

Methemoglobinemia can be detected by observing cyanosis of mucous membranes or brownish discoloration of blood, as well as specific testing for methemoglobin. The latter test is available at many reference laboratories. Methemoglobinemia should be suspected if Heinz body formation is seen. The animal may appear more hypoxic than the PCV suggests. The gross appearance of blood is often the first suggestion of methemoglobinemia. Blood with methemoglobinemia is darker and browner than normal and may not turn red when exposed to air. Placing a drop of blood on white filter paper allows one to look for the brown color more easily. The animal's mucous membranes may appear darker or cyanotic if more than 30% of hemoglobin is affected. Causes of canine methemoglobinemia include benzocaine and acetaminophen administration. Causes of feline methemoglobinemia include benzocaine (Cetacaine), ketamine, acetaminophen, phenacetin, methylene blue, DL-methionine, and phenazopyridine. Toxic substances (e.g., benzocaine, acetaminophen, methylene blue) may cause Heinz body anemia, methemoglobinemia, or both.

Blood Parasites

Haemobartonella felis (Mycoplasma haemofelis)

Haemobartonella felis (e.g., feline infectious anemia) infection can be diagnosed by blood smear evaluation or by use of a commercially available PCR test (Messick et al, 1998). The PCR test is more sensitive test for detection of feline hemobartonellosis than blood smear analysis and appears also to detect canine hemobartonellosis. Blood smear evaluation is insensitive. Stain precipitate is commonly present, is coccoid in shape, and is frequently confused with *H. felis* organisms, resulting in misdiagnosis. To differentiate *H. felis* from stain precipitate, at least two distinct ring forms of *H. felis* should be identified (see Color Plate 1D). A ring form is like a lowercase letter *o* with a dark exterior ring and a pale interior (see Figure 2-9).

Ring forms are at the smallest resolution that can be distinguished with a good microscope with proper condenser settings. Parasitemic episodes (e.g., *H. felis* visible on RBCs) are frequently present for relatively short periods (i.e., 1 to 2 days) separated by periods when few or no organisms are found. Therefore smears should be evaluated on several consecutive days to improve the likelihood of diagnosis.

Obtaining fresh, nonanticoagulated, capillary blood by pricking the ear with a lancet and immediately making a thin blood smear provides a higher concentration of parasitized RBCs. The parasites may fall off RBCs in EDTA blood. Splenomegaly is common in feline infectious anemia because splenic macrophages remove damaged RBCs and organisms. *Mycoplasma haemofelis* is the current name for *Haemobartonella felis*.

Haemobartonella canis (Mycoplasma haemocanis)

Haemobartonella canis principally causes anemia in dogs that have undergone splenectomy. It may occur in carrier dogs after splenectomy or in dogs that have previously undergone splenectomy after they contract infection via blood transfusion or tick bite. Glucocorticoid therapy may produce a functional splenectomy and thereby predispose to *H. canis* infection. *H. canis* appears as distinct chains of cocci on RBCs. The linearly arranged cocci must be differentiated from stain precipitate. *Mycoplasma haemocanis* is the current name for *Haemobartonella canis*.

Babesia Canis

Babesia canis can produce severe anemia in dogs. Both acute intravascular hemolysis and extravascular hemolysis are seen. Hemoglobinuria is common. Transmitted by ticks, it is often accompanied by *Ehrlichia canis* and other infections. *Babesia* is seen more frequently in racing greyhound dogs. The diagnosis is made by demonstrating intraerythrocytic piriform (i.e., pear shaped or teardrop shaped) organisms on blood smears or by serology. Capillary blood has a higher concentration of parasitized RBCs, so "ear prick" blood samples are recommended for immediately made blood smears. *Babesia gibsoni* is smaller than *B. canis*, but both

organisms are large enough to be readily seen microscopically. *B. gibsoni* is so common in pit bull terriers that they should not be used as blood donors.

Cytauxzoon Felis

Cytauxzoon felis is a fatal, tick-transmitted disease of cats. It is diagnosed most consistently at necropsy by the finding of large schizonts in endothelial cells of the lungs, liver, spleen, and lymph nodes. Endemic areas include Missouri, Oklahoma, Arkansas, Mississippi, Georgia, Florida, and Louisiana. Small piriform or safety pin–shaped organisms in RBCs are found on blood smears in less than 50% of affected cats. These bodies measure 1 to 5 μm and have a small, peripherally located nucleus. Clinical signs include icterus, depression, anorexia, fever, and dehydration.

Zinc Toxicity

Zinc-induced hemolytic anemia results from ingestion of zinc-containing objects (e.g., zinc nuts from portable kennels or pennies [which are small U.S. coins]) by dogs. Zinc forms soluble salts in gastric acid and is absorbed. Zinc toxicity causes intravascular hemolysis. Clinical signs include severe anemia, icterus, leukocytosis, vomiting, and diarrhea. Diagnosis is confirmed by detection of zinc-containing objects in the stomach by radiographic examination and increased serum zinc concentration (normal canine serum zinc = 0.6 to 2 mg/kg) (see Chapter 17). An increase in spherocytic RBCs occurs in zinc toxicity. It is unclear whether these are spherocytes indicating immune damage or pyknocytes resulting from oxidative damage. Treatment of canine RBCs with zinc chloride induces IgG binding; therefore zinc-induced hemolysis may be a secondary form of IMHA (Christian et al, 1995).

Hypophosphatemia

Hypophosphatemia causes hemolytic anemia in dogs and cats. In dogs hemolysis can occur when serum phosphorus concentration is less than 1.0 mg/dl. However, in cats, hemolysis may occur with less severe hypophosphatemia (i.e., < 2.5 mg/dl) (Justin and Hohenhaus, 1995). Hypophosphatemia may be anticipated when refeeding cats with hepatic lipidosis. Hypophosphatemia may be a complication of insulin therapy. Diagnosis of hypophosphatemia (see Chapter 8) in hemolytic anemia is complicated by a common laboratory error. Conjugated bilirubin in icteric samples interferes with the direct inorganic phosphorus (PO_4) assay (Alvarez, Whalen, and Scott, 1993). Not all routine PO_4 assays are affected by icterus. The PO_4 concentration may appear falsely decreased or even too low to measure in very icteric samples because of this laboratory error, whereas the PO_4 could actually be normal or high. If in doubt, the clinician can verify PO_4 concentration in icteric samples using inductively coupled plasma emission spectrometry methods modified for serum used in toxicology laboratories.

Pyruvate Kinase Deficiency

Pyruvate kinase (PK) deficiency in dogs is an autosomal recessive genetic disease causing severe and persistent extravascular hemolysis. Characteristic features include moderate-to-severe anemia (i.e., PCV = 18% to 25%) with marked reticulocytosis (i.e., 25% to 45%) possibly caused by rapid and persistent RBC turnover and to splenomegaly that occurs in young basenji, West Highland white terrier, or beagle dogs. Affected dogs die by 3 years of age, and many dogs have terminal myelofibrosis and osteosclerosis. PK-deficient RBCs have inefficient energy production via the glycolytic pathway and thus have a shortened life span.

Other Hereditary Hemolytic Anemias

Phosphofructokinase deficiency occurs in English springer spaniels. Episodic hemoglobinuria is common, and splenomegaly, icterus, or both may be present. Intravascular hemolysis is precipitated by respiratory alkalosis associated with stress-induced hyperventilation, because residual phosphofructokinase activity in affected dogs is inhibited by alkalotic conditions. Diagnosis is confirmed by analysis of RBC phosphofructokinase activity after ruling out common causes of hemolytic anemia (Harvey and Giger, 1984).

Familial nonspherocytic hemolytic anemia has been described in poodles and beagles. In poodles the cause was not determined, but PK activity was not evaluated. It was fatal by as early as 3 years of age. Myelofibrosis, osteosclerosis, and widespread hemosiderosis were noted at necropsy, as in dogs with PK deficiency. Hereditary nonspherocytic hemolytic

anemia in beagles was studied extensively without identifying a cause. The anemia was not severe or fatal and lacked hemosiderosis, myelofibrosis, or osteosclerosis.

Dwarfism in Alaskan malamutes has been associated with stomatocytosis. Stomatocytes are RBCs with a mouth-shaped central pallor. RBC are macrocytic and hypochromic. The MCHC was consistently less than 30 g/dl. Compared with age-matched normal Alaskan malamutes, the dwarfs were not anemic.

Miniature schnauzers have stomatocytosis as an autosomal recessive trait but lack clinical signs of anemia. A miniature schnauzer-beagle dog had inherited stomatocytosis and prominent macrocytosis, apparently the result of RBC swelling (see Figure 3-4). It had a PCV of 48% but had a mild reduction in RBC numbers (i.e., $5.01 \times 10^6/\mu l$) and hemoglobin concentration (12.5 g/dl) (Brown, 1994). RBC survival is only slightly shortened. Stomatocytosis also occurs in Drentse patrijshond breed familial stomatocytosis-hypertrophic gastritis.

Hereditary elliptocytosis in dogs is associated with a deficiency of membrane protein 4.1. RBC morphology was characterized by elliptocytosis and poikilocytosis, but anemia was not present.

Hemolytic anemia with increased osmotic fragility has been described in Abyssinian and Somali cats. Affected cats have a moderate-to-severe direct Coombs'-negative hemolytic anemia. RBC osmotic fragility is markedly increased.

NONREGENERATIVE ANEMIA

Nonregenerative anemia is usually normocytic normochromic and lacks diagnostic changes in RBC morphology. Many nonregenerative anemias are only mild-to-moderate and are secondary complications of systemic diseases, especially inflammatory and neoplastic diseases. Diagnostic efforts should be aimed at the primary disease. Anemia of renal failure can become severe in late stages of disease. Severe nonregenerative anemia may exist separately or be part of generalized bone marrow suppression that results in pancytopenia or bicytopenia. Likely causes are listed in Box 3-1.

Diagnostic Approach

One should first determine if leukopenia, thrombocytopenia, or both are present in addition to nonregenerative anemia (see Box 3-2). Pancytopenia (i.e., decreased RBCs, leukocytes, platelets) or bicytopenia (i.e., two of the three cell types depleted) usually indicates disease within the bone marrow, which is best diagnosed by a bone marrow biopsy and aspiration (see Chapter 2; also discussed later) (Weiss, 1992).

If only anemia is present, one should check the RBC indices, RBC cytogram and histograms, and blood smear to classify it as normocytic normochromic, microcytic hypochromic, or macrocytic normochromic. *Normocytic normochromic anemia* is the most frequent. If the anemia is mild-to-moderate, causes of secondary bone marrow suppression should be evaluated. Severe nonregenerative anemias are frequently associated with nonregenerative immune-mediated hemolytic anemias or pure RBC aplasia. *Microcytic hypochromic anemia* indicates iron deficiency (discussed later). *Macrocytic normochromic anemia* (discussed earlier) without reticulocytosis in cats is frequently associated with FeLV-induced myeloproliferative disorders. If a cause for the anemia cannot be identified, a bone marrow evaluation is indicated.

Secondary Anemia

Anemia of Inflammatory Diseases

Anemia of inflammatory or neoplastic diseases is the most common cause of nonregenerative anemia. Mild-to-moderate normocytic normochromic nonregenerative anemia that does not become severe, low serum iron, and increased iron in bone marrow and other tissues is typical. Inflammatory changes in the CBC are discussed in Chapter 4. During inflammation, macrophages release inflammatory cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor, which initiate several processes including fever. Inflammatory cytokines cause iron to be sequestered in macrophages, which reduces serum iron and restricts the availability of iron to developing rubricytes, thus limiting erythropoiesis. RBC life span is also reduced.

Anemia of Chronic Renal Disease

The diagnosis of anemia of chronic renal disease is confirmed by finding nonregenerative anemia associated with renal failure (see Chapter 7). The mechanism of the anemia is more complex than a relative deficiency

of erythropoietin. Ineffective erythropoiesis, a shortened RBC life span, and blood loss may contribute to the anemia. This type of anemia responds well to erythropoietin therapy.

Anemia of Chronic Hepatic Disease

Anemia of chronic hepatic disease may have multiple causes (see Chapter 9). Abnormal lipid metabolism may cause altered RBC shapes (i.e., acanthocytes, budding fragmentation) and a shortened RBC life span. Marked poikilocytosis is a frequent finding in cats with hepatic lipidosis and inflammatory liver disease. Coagulation defects, caused by reduced hepatic synthesis of coagulation and anticoagulant factors, may cause hemorrhage. Decreased hepatic function can lead to deficiencies of nutrients needed for hematopoiesis. Microcytosis is frequent finding in dogs with a portosystemic shunt (PSS). Dogs with a PSS often have a "functional iron deficiency" with increased hepatic iron but usually low serum iron, normal total iron–binding capacity (TIBC), and low percentage saturation (Table 3-4) (Meyer and Harvey, 1994).

Hypothyroidism and Hypoadrenocorticism

Anemia secondary to hypothyroidism and hypoadrenocorticism usually is mild and clinically insignificant. Evaluation of endocrine disorders is discussed in Chapter 8.

Severe Nonregenerative Anemia

Causes of severe nonregenerative anemia without accompanying leukopenia or thrombocytopenia include nonregenerative immune-mediated anemias (NRIMAs) pure red blood cell aplasia (PRCA), iron deficiency anemia, chronic renal failure, FeLV and feline immunodeficiency virus (FIV) infection, myelodysplastic syndromes with refractory anemia (MDS-RA), and myelodysplastic syndromes with erythroid predominance (MDS-Er).

Nonregenerative Immune-Mediated Hemolytic Anemia

NRIHA is a form of IMHA in which erythropoiesis is inhibited. This may be the result of destruction of erythroid precursor cells by autoantibodies; concurrent myelofibrosis, myelodysplastic syndromes, and secondary hemophagocytic syndromes; or bone marrow necrosis. Bone marrow may have erythroid hyperplasia or erythroid hypoplasia. In some dogs, a maturation arrest is present, indicating that the more mature cells (e.g., metarubricytes, later-stage rubricytes) are being destroyed.

Pure Red Cell Aplasia

PRCA is a severe nonregenerative anemia characterized by bone marrow that has few or no erythroid precursors, yet normal-appearing myeloid and megakaryocytic lines. The cause is believed to be immune mediated. Antibodies against erythroid precursor cells were found in four of eight dogs with PRCA, and the Coombs' reaction sometimes is positive. Most dogs respond to steroidal or nonsteroidal immunosuppressive therapy. *Parvovirus* infection has also been associated with PRCA.

Myelodysplastic Syndrome with Refractory Anemia

MDS-RA is caused by an acquired genetic defect in hematopoietic stem cells. Anemia is

TABLE 3-4. Canine Iron Profiles in Iron Deficiency Anemia, Anemia of Inflammatory Disease, and Portosystemic Shunts

	IRON DEFICIENT* MEAN (RANGE)	NORMAL* MEAN (RANGE)	AID† MEAN ± SD	NORMAL† MEAN ± SD	PSS‡ MEAN (RANGE)
Serum iron (µg/dl)	30 (8-60)	149 (84-233)	62 ± 14	113 ± 8	77 (24-163)
TIBC (µg/dl)	387 (234-659)	391 (284-572)	193 ± 28	309 ± 36	377 (302-452)
UIBC (µg/dl)	357 (216-633)	243 (142-393)			300 (184-379)
Saturation (%)	8 (2-19)	39 (20-59)			21 (6-45)

*Harvey, French, and Meyer, 1982.
†Feldman, Kaneko, and Farver, 1981.
‡Meyer and Harvey, 1994.
TIBC, Total iron-binding capacity; *UIBC*, unbound iron-binding capacity; *AID*, anemia of inflammatory disease; *PSS*, portosystemic shunt.

a moderate-to-severe, normocytic normochromic, and nonregenerative. The disorder results in production of abnormal erythroid precursor cells that have atypical morphologic features and die before being released into the blood. Bone marrow is usually normally cellular or hypercellular and has erythroid hyperplasia. Morphologic alterations that may be observed in bone marrow erythroid precursor cells include, binucleated RBC precursors, asynchronous maturation of cytoplasm and nucleus, macrocytes, microcytes, megaloblasts, and Howell-Jolly bodies. Among the limited number of affected dogs described, the anemia appears to respond well to erythropoietin therapy and affected dogs have prolonged survival.

Myelodysplastic Syndrome with Erythroid Predominance

MDS-Er is similar to MDS-RA but has increased numbers of rubriblasts in the bone marrow. It is unclear if MDS-Er and MDS-RA are separate disorders or variations of the same disorder.

Congenital Myelodysplastic Syndromes

Congenital causes of MDS include congenital dyserythropoiesis, polymyopathy, and cardiac disease in English springer spaniels, vitamin B_{12} malabsorption in giant schnauzers, and poodle macrocytosis. Congenital dyserythropoiesis, polymyopathy, and cardiac disease in English springer spaniels is characterized by moderate anemia with spherocytes, schizocytes, dacryocytes, codocytes, and vacuolated RBCs seen in blood smears. Bone marrow is characterized by erythroid hyperplasia with dyserythropoiesis (i.e., binucleation and abnormal mitotic figures). These congenital causes of MDS are positioned in the text with the previously discussed types of MDS.

Vitamin B_{12} malabsorption in giant schnauzers is an autosomal recessive disorder characterized by chronic nonregenerative anemia and neutropenia. Dysplastic features in the blood include macrocytes, schizocytes, acanthocytes, elliptocytes, keratocytes, hypersegmented neutrophils, and giant platelets. Dysplastic features in bone marrow include giant band neutrophils and asynchronous maturation in the erythroid series. Clinical signs resolve with parenteral administration of vitamin B_{12}.

Poodle macrocytosis is a condition of toy and miniature poodles that is characterized by macrocytosis in the blood and marked dyserythropoiesis in the bone marrow. Dysplastic features in bone marrow include megaloblasts, binucleated erythroid cells, nuclear fragmentation, multiple Howell-Jolly bodies, and atypical mitotic figures. Despite the very abnormal morphology, the dogs are not anemic and have no clinical signs.

Iron Deficiency Anemia

Iron deficiency anemia is not uncommon. Harvey, French, and Meyer (1982) documented it in 11% of anemic dogs in Florida. Reticulocytosis and polychromasia occurred in half of affected dogs; therefore clinicians should not exclude iron deficiency in slightly to moderately regenerative anemia. The cause is usually persistent blood loss (e.g., hookworms, fleas, bleeding intestinal neoplasms). Blood loss anemias are initially strongly regenerative, then progress to nonregenerative as iron deficiency worsens. Anemia varies from mild to severe. Poikilocytosis with fragmentation and hypochromasia is typical. Thrombocytosis can be marked with many large platelets.

Hypoproteinemia, when present, supports recent and severe external bleeding; however, PP losses are replaced much faster than RBCs in the body, so it may be normal in chronic bleeding. Puppies normally have lower PP concentrations than adults do; therefore clinicians should use age-matched reference values when evaluating anemic puppies. Puppies have lower PCV and higher reticulocyte values than adults. Puppies become iron deficient more rapidly than adults, because of their rapid growth and maximal erythropoiesis.

A CBC usually identifies iron deficiency anemia (see Color Plate 2A). Microcytic (i.e., MCV < 60 fl), hypochromic (i.e., MCHC < 32 g/dl) RBCs are highly diagnostic for iron deficiency. Newer automated hematology cell counters are more sensitive in detecting small numbers of microcytic hypochromic RBCs (see Figure 2-4) than MCV and MCHC, which are mean values for all RBCs (Tvedten, 1993a). Exceptions include Japanese Akita and Shibas, dogs that normally have small RBCs. Microcytosis and decreased serum iron occur often in dogs with PSSs and resemble iron deficiency (discussed earlier).

The serum iron profile is the best proof of iron deficiency and can provide confidence

in the diagnosis. The owner and veterinarian may become discouraged at how slowly PCV returns to normal. Expected iron profile values in iron deficiency are in Table 3-4, and are compared with iron values in dogs with anemia of inflammatory disease that commonly causes low serum iron (Table 3-5) (Feldman, Kaneko, and Farver, 1981).

TIBC reflects transferrin, the serum protein that binds iron. Serum iron measures the iron bound to transferrin. Unsaturated iron-binding capacity (UIBC) measures the additional amount of iron that transferrin can bind. TIBC is the sum of serum iron plus UIBC. Percentage saturation is serum iron divided by TIBC (normal = 20% to 60%). Percentage saturation indicates availability of iron for erythropoiesis. Hemoglobin synthesis is impaired if percentage saturation is less than 20%. Hemosiderin is not detectable in bone marrow samples of iron deficient dogs.

Iron deficiency anemia associated with microcytosis is common (i.e., 70%) in 5-week-old kittens (Weiser and Kociba, 1983). By 7 weeks of age, the kittens stopped producing microcytic cells. The iron deficiency is probably the result of the low iron content of all-milk diets.

Feline Leukemia and Feline Immunodeficiency Virus Infection

FeLV and FIV infection are the major causes of feline nonregenerative anemia. Macrocytosis (i.e., MCV > 52 fl) without increased reticulocytes is an indication of FeLV infection.

Pancytopenia or Bicytopenia

Pancytopenia or bicytopenia usually reflects poor marrow production of two or three cell lines, respectively, and suggests marrow disease. RBCs have the longest life span, so nonregenerative anemia may be detected later than thrombocytopenia and leukopenia.

Aplastic Pancytopenia and Myelofibrosis

Diagnosis of aplastic pancytopenia and myelofibrosis is confirmed by histopathologic examination of bone marrow core biopsies (see Chapter 2). Bone marrow aspirates in aplastic pancytopenia and myelofibrosis are poorly cellular, perhaps only retrieving fat, so the aspirate may seem to be a poor sample. Bone marrow biopsies allow evaluation of marrow architecture necessary to confirm marrow hypocellularity and replacement by fat or fibrous tissue. If hemic tissue is replaced by fatty tissue, the condition is best called *aplastic pancytopenia* (also *aplastic anemia* or *fatty bone marrow*). Possible causes of aplastic pancytopenia include drug toxicities (e.g., estrogen, phenylbutazone, chemotherapeutic agents, sulfadiazine, quinidine, thiacetarsemide, griseofulvin), *E. canis, Parvovirus,* and immune-mediated processes.

Myelofibrosis is characterized by replacement of hemic tissue by fibrous connective tissue, collagen, or reticulin fibers. Myelofibrosis can be primary or secondary. Secondary myelofibrosis may occur after toxic marrow injury or can result from overproduction of erythropoietin or thrombopoietin. Therefore myelofibrosis tends to be associated with immune-mediated anemias and thrombocytopenias and congenital hemolytic anemias. Primary myelofibrosis is a chronic myeloproliferative disorder of erythroid, myeloid, and megakaryocytic cell lines. Growth factors released by abnormal megakaryocytes are implicated as the cause of fibroblast proliferation.

Drug-Induced Hematologic Dyscrasia

Drugs reported to cause bone marrow dyscrasia in dogs include estrogens, phenylbutazone, meclofenamic acid, sulfonamides, quinidine,

TABLE 3-5. Comparison of Selected Parameters in Anemia of Chronic Inflammation and Iron Deficiency Anemia

	ANEMIA OF INFLAMMATORY DISEASE	IRON DEFICIENCY ANEMIA
RBC indices	Normocytic normochromic	Microcytic hypochromic
Serum iron	Low	Low
Total iron-binding capacity	Usually decreased	Usually normal
Bone marrow iron	Abundant to increased	Absent
Serum ferritin	High	Low
Inflammation	Present	Need not be present

RBC, Red blood cell.

chloramphenicol, cephalosporins, chemotherapeutic agents, captopril, tranquilizers, trimeprazine tartrate, mitoxantrone, thiacetarsemide, albendazole, phenobarbital, and phenothiazine. Drugs reported to cause bone marrow dyscrasia in cats include chemotherapeutic agents, chloramphenicol, griseofulvin, propylthiouracil, and methimazole.

Estrogen Toxicity

Canine estrogen toxicity may cause bone marrow destruction, leaving a fatty marrow and aplastic pancytopenia. During the first 3 weeks, however, thrombocytopenia, leukocytosis, and mild but progressive anemia typically characterize the CBC. Total leukocyte counts may exceed 100,000 WBCs/μl (Figure 3-5). The cause of the leukocytosis is uncertain. After 3 weeks, pancytopenia may occur. Testicular tumors, usually Sertoli's cell tumors, may also cause pancytopenia or bicytopenia. Increased serum estradiol concentrations cannot be consistently documented. The PCV in anemias associated with testicular tumors have ranged from 6% to 38%. The reticulocytes were decreased in number or only slightly increased. Thrombocytopenia varied from 3000 to 93,000/μl.

Sulfadiazine Toxicity

Doberman pinschers appear to be predisposed to sulfadiazine-induced hematologic dyscrasia. Affected dogs have anemia, leukopenia, thrombocytopenia, or a combination thereof with variable polyarthritis, lymphadenopathy, polymyositis, glomerulonephritis, and retinitis. Affected Doberman pinschers and other affected dogs usually recover after discontinuation of the drug.

Phenylbutazone Toxicity

Hematologic dyscrasias associated with phenylbutazone toxicity include severe aplastic pancytopenia; transient neutropenia, with or without thrombocytopenia; thrombocytopenia; PRCA; and hemolytic anemia. Dogs with phenylbutazone-induced aplastic pancytopenia rarely recover.

Ehrlichiosis

Canine ehrlichiosis is caused by different *Ehrlichia* species. Severity of hematologic changes varies with different types of *Ehrlichia*. See Chapter 15 for serologic diagnosis. *E. canis* invades monocytes and lymphocytes (i.e., tropical canine pancytopenia). Acute infection consistently causes thrombocytopenia. Antiplatelet antibodies are consistently present during *E. canis* infection, and some dogs are direct Coombs' positive. Chronic ehrlichiosis can cause nonregenerative anemia, thrombocytopenia, and leukopenia. Hematologic dyscrasia may result from bone marrow suppression and destruction of blood cells.

Granulocytic ehrlichiosis is caused by *E. ewingii* and *Anaplasma phagocytophila* in the United States. Swedish granulocytic *Ehrlichia* is common in Sweden. It is closely related to E. equi and A. phagocytophila. Kits for detection of E. equi are used for detection of dogs with positive titers in Sweden. *Ehrlichia* inclusions in neutrophils are seen in Swedish granulocytic ehrlichiosis 4 to 14 days after infection and persist 4 to 8 days (Lilliehook, Egenvall, and Tvedten, 1998). Hematologic changes are maximal during parasitemia, transient, and include mild nonregenerative anemia, moderate thrombocytopenia, lymphopenia, neutropenia, and eosinopenia.

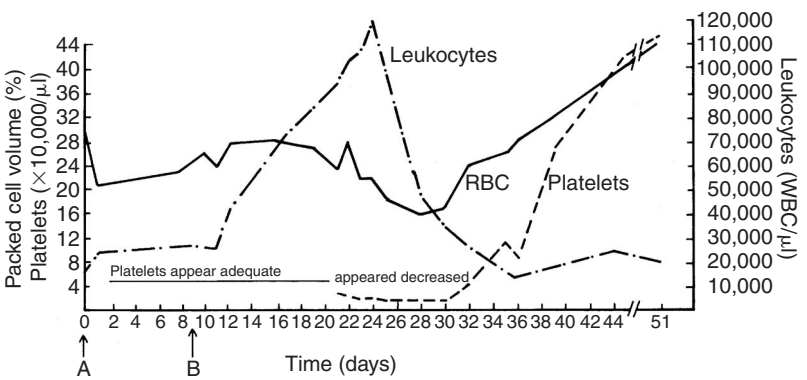

FIGURE 3-5. Estrogen toxicity in a dog. The transient, time-related hematologic changes in a dog with an excessive dose of estradiol included bicytopenia consisting of thrombocytopenia and nonregenerative anemia and a profound leukemoid response. ***A,*** A bleeding episode occurred. ***B,*** Estradiol was given. *RBC,* Red blood cell; *WBC,* white blood cell.

Serum iron and TIBC decrease, suggesting anemia of inflammation.

Feline Leukemia Virus

In most (90%) FeLV infected cats, erythroid parameters are altered. FeLV is commonly associated with cases of feline nonregenerative anemia. One pattern suggestive of FeLV infection is macrocytosis (i.e., MCV > 52 fl) without increased reticulocytes.

Feline Immunodeficiency Virus

Cats infected with FIV often have nonregenerative anemia. Neutropenia may be concurrent in some patients (Flemming et al, 1991). Rarely, hemolytic anemia, possibly caused by the virus and not secondary *Haemobartonella* infection, occurs.

Parvovirus

Parvovirus infection in both dogs and cats (feline panleukopenia) destroys bone marrow precursor cells. Infectious panleukopenia causes severe, absolute granulocytopenia and lymphopenia. Anemia is mild and may not be detected because PCV may not decrease below the reference range and animals may be dehydrated. Anemia is usually nonregenerative, but an increase in reticulocytes may occur during the recovery period.

Myelodysplastic Syndromes and Leukemias

Although MDS-RA and MDS-Er result in nonregenerative anemias, myelodysplastic syndromes with excess myeloblasts (MDS-EB) typically cause pancytopenia. Dysplastic features are seen in two or mores cell lines in the bone marrow. MDS-EB may progress to acute myelogenous leukemia. Acute leukemias are discussed in Chapter 4.

Macrophage Proliferative Disorders

Macrophage and histiocyte proliferative disorders may be malignant or benign. Malignant disorders include malignant histiocytosis and disseminated histiocytic sarcoma. Histiocytic disorders (i.e., systemic histiocytosis, malignant histiocytosis) are closely associated with Bernese mountain dogs. Malignant histiocytosis has aggressive proliferation of very atypical cells in a variety of tissues and has pancytopenia. Benign conditions have been termed *hemophagocytic syndromes* or *hemophagic histiocytosis*. Hemophagocytic syndrome is characterized by proliferation of hemophagocytic macrophages, which results in pancytopenia in the blood. Hemophagocytic syndromes can be idiopathic or occur secondary to IMHA, immune-mediated thrombocytopenia, myelodysplastic syndromes, histiocytic disorders, or neoplasia.

Excessive proliferation of macrophages and histiocytes with active erythrophagocytosis can be so extreme as to cause persistent, nonregenerative, severe anemia. Hemophagic histiocytosis is uncommon and has excessive, well-differentiated histiocytes found most predominantly in the bone marrow and also liver, lymph nodes, and spleen. These macrophages are heavily laden with hemosiderin and with phagocytosis of mature and nucleated erythrocytes. Hemophagic syndromes with excessive erythrophagocytosis must be differentiated from the more common erythrocyte breakdown in normal macrophages seen in hemolytic anemias or hemosiderin accumulation in macrophages seen in nonregenerative anemias. Occasional mast cell neoplasms have prominent erythrophagocytosis associated with anemia.

BLOOD TRANSFUSION AND BLOOD TYPING

Blood transfusion may be a life-saving treatment, but it is best avoided unless the anemia is severe or causing clinical signs. Blood contains many cell types and proteins that are highly immunogenic and may sensitize the patient to later reactions. Indications for transfusion include when the PCV is less than 11% in cats and less than 13% in dogs and the presence of clinical signs including tachycardia, poor-quality pulse, lethargy, and weakness. In general, clinical signs are more important than is PCV in determining the need for transfusion. Donors' blood should be cross-matched to find the blood least likely to cause transfusion reactions. Acute transfusion reactions include fever, hypersensitivity reactions, shock, hemolysis, and disseminated intravascular coagulation. Chronic transfusion reactions include decreased life span of transfused cells or acquired immune reactions to the recipient's own cells, such as posttransfusion purpura. In posttransfusion purpura, repeated transfusions can lead to a sudden destruction of the recipient's own platelets.

Ideally, the blood type of the patient and donor animal should be determined (see Appendix I). Alternatively, the clinician should use donor dogs that are negative for dog erythrocyte antigen (DEA) 1.1, 1.2, and 7 (the RBC antigens most likely to cause transfusion reactions). Cross matches should always be performed before transfusion, because at least eight blood groups have been described for dogs. Three feline blood groups have been described and designated A, B, and AB. As little as 5 ml of type A blood transfused into a type B cat can cause a fatal transfusion reaction. The incidence of type B is low in the United States, but some purebred cats (e.g., Abyssinian, Himalayan, British shorthair) have a higher incidence of type B. In-office kits to identify DEA 1.1 and feline A, B, and AB are available.*

POLYCYTHEMIA

Polycythemia is an increase in PCV, hemoglobin, or RBC count. Polycythemia is absolute polycythemia (i.e., increased erythroid mass associated with increased bone marrow production of RBCs) or relative polycythemia (i.e., increased PCV because of decreased plasma volume or splenic contraction). Neurologic signs, such as seizures and collapse, may occur when the PCV exceeds 70% (probably because of hyperviscosity of blood causing poor perfusion of the brain).

Relative Polycythemia

Relative polycythemia is not an absolute increase in the RBC mass in the body. It is usually the result of decreased vascular fluid (i.e., dehydration, hemoconcentration, hypovolemia). Another cause is splenic contraction, which is a distribution shift of a concentrated bolus of RBCs out of the spleen. In relative polycythemia caused by hemoconcentration or dehydration, the PCV should return to normal after fluid replacement (Figure 3-6). Hemoconcentration is frequently accompanied by a concurrent increase in PP concentration, clinical syndromes that cause fluid loss (e.g., diarrhea, vomiting), and other evidence of hypovolemia. The PCV and PP concentration are clinically useful indicators of hemoconcentration but are insensitive indicators of dehydration because of wide reference ranges. Elevation of PCV and PP concentration from the mean reference value can show a trend toward hemoconcentration.

Relative polycythemia of splenic contraction may be more difficult to recognize because one cannot consistently predict the degree of splenic contraction or relaxation. Splenic contraction is expected after exercise, excitement, or fear. The PCV should return to normal within 1 hour after the animal calms down.

Absolute Polycythemia

Absolute polycythemia is subdivided into primary and secondary conditions. The reader should note difference between *secondary* and *relative* polycythemia.

Primary Absolute Polycythemia

Primary absolute polycythemia is called *polycythemia vera* or *primary erythrocytosis*, which is an uncommon chronic myeloproliferative disorder. The disorder is caused by uncontrolled proliferation of an defective clone of erythroid cells. Primary absolute polycythemia is usually diagnosed by excluding other causes of polycythemia. The PCV usually remains at 70% to 80% despite fluid therapy. Serum erythropoietin concentration is not increased. Primary polycythemia is complicated by hyperviscosity that becomes increasingly more severe as the PCV exceeds 60%.

Secondary Absolute Polycythemia

Secondary polycythemia is divided into appropriate and inappropriate forms. *Appropriate secondary polycythemia* is caused by increased production of erythropoietin as the result of hypoxia (e.g., pulmonary or cardiac disease, living at high altitude). Arterial blood gas analysis documents hypoxemia (i.e., low partial pressure of oxygen). Serum erythropoietin concentrations should be increased. Additional evidence of absolute polycythemia may include documentation of erythroid hyperplasia in bone marrow samples or slight polychromasia in blood samples despite polycythemia.

Inappropriate secondary polycythemia may result from renal diseases in which excessive erythropoietin secretion occurs. Polycythemia occurs in dogs with renal cell carcinoma, renal lymphosarcoma, and chronic pyelonephritis. Cases have had PCVs

*Rapid Vet-H, DMS Laboratories Inc., Flemington, NJ.

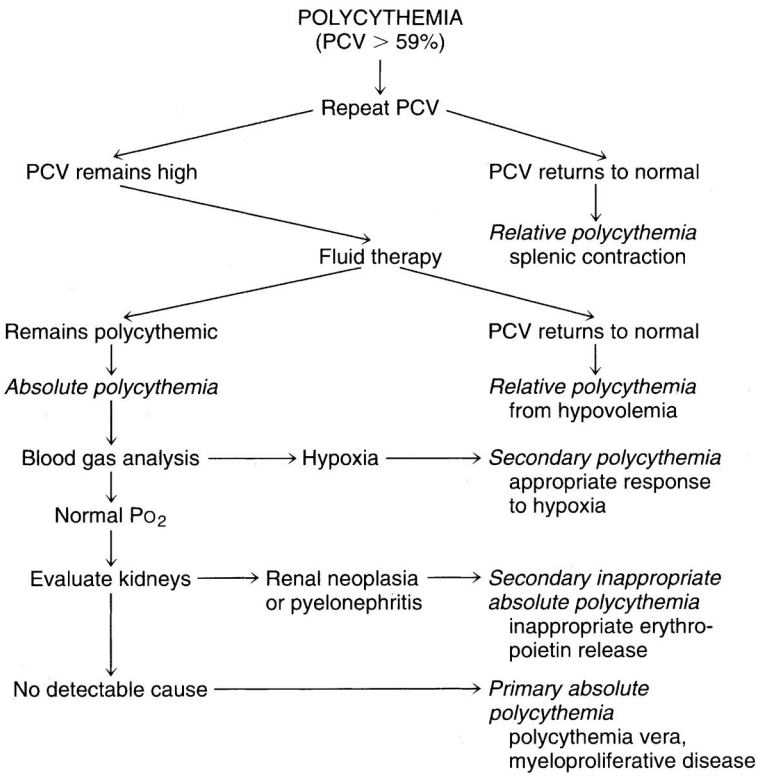

FIGURE 3-6. Diagnostic approach to polycythemia. The italicized common conclusions are made using the various procedures listed. Primary polycythemia is diagnosed by exclusion of causes listed above it (i.e., when splenic contraction is unlikely, the hydration status is normal; no hypoxia is found from pulmonary, cardiac, or hemoglobin disorders; and the kidneys are normal). *PCV*, Packed cell volume.

of 64% to 81%. Serum erythropoietin concentration, which is normally not detectable in dogs, is increased (i.e., 0.1 to 0.3 IU/ml). After removal of the diseased kidney, the PCV and erythropoietin concentration should return to normal.

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4

Rose E. Raskin, Kenneth S. Latimer, and Harold Tvedten

Leukocyte Disorders

BASIC LEUKOCYTE CONCEPTS

Leukogram

Leukocyte responses are evaluated by component parts of the leukogram, including total leukocyte (white blood cell [WBC]) count, differential leukocyte count, absolute numbers of specific leukocytes per microliter of blood, and examination of leukocyte morphology on the Romanowsky's-stained (e.g., Wright's-stained) blood smear.

Hematologic techniques are described in Chapter 2. The leukocyte differential count is the relative percentages of various leukocyte types (i.e., segmented neutrophils [segs], band neutrophils, lymphocytes, monocytes, eosinophils, basophils) in the stained blood smear. Absolute leukocyte counts are the numbers of each type of leukocyte per microliter of blood.

The leukogram is used to monitor the patient's health status, to construct a differential diagnosis, to evaluate a patient's response to treatment, or to suggest a prognosis. Although changes in the leukogram are seldom pathognomonic for a given disease, they do identify and characterize several disease processes and indicate trends suggesting development or resolution of illness (Latimer, 1995). The leukogram does not normally document sepsis, identify specific causative agents, or pinpoint location of inflammation. However, characteristic changes in the leukogram identify presence of inflammatory disease and characterize its severity. Toxic changes and severity of inflammation may suggest sepsis, which may be confirmed and localized by cytology, microbiologic culture, serum chemistries, radiographs, ultrasonography, surgical biopsy, or serology. Any one or a combination of these tests may localize the disease process to a tissue or organ system or provide a definitive diagnosis.

This chapter is concerned with understanding and interpreting leukogram abnormalities. Basic leukocyte concepts are reviewed to facilitate understanding the leukocyte response and to avoid common interpretive errors. Supplemental information regarding leukogram interpretation may be found in other textbooks (Duncan, Prasse, and Mahaffey, 1994; Latimer, 1995; Meyer and Harvey, 1998; Feldman, Zinkl, and Jain, 2000).

Absolute Versus Relative Leukocyte Values

Use of absolute WBC numbers allows more consistent evaluation of leukogram responses than using relative percentages (Figure 4-1). For example, a WBC count of 10,000 leukocytes/μl with 65% segs has 6500 segs/μl. The 6500 segs/μl is normal, but 65% segs are not always normal. A WBC count of 1000 leukocytes/μl with 65% segs indicates severe neutropenia (i.e., 650 segs/μl). A WBC count of 50,000 leukocytes/μl with 65% segs indicates neutrophilia (i.e., 32,500 segs/μl).

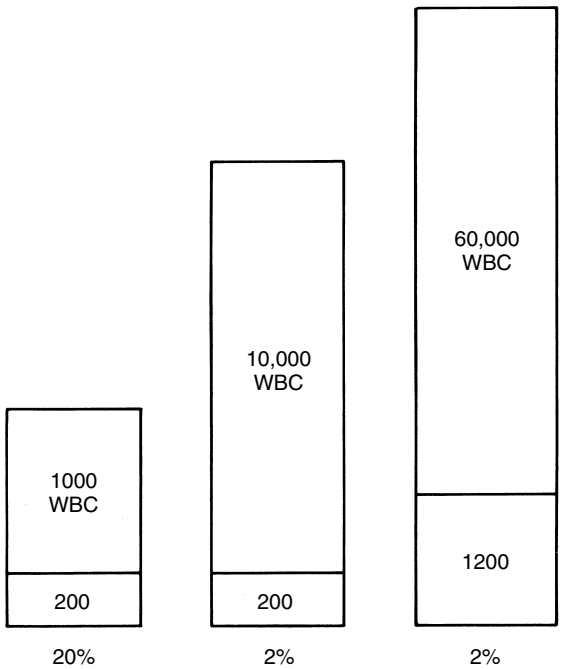

FIGURE 4-1. Relative and absolute leukocyte counts. The bottom chamber of each bar indicates the absolute number of band neutrophils, and the percentage below the bars indicates the relative percentage of band neutrophils. The relative change between the first and second bar (i.e., 20% to 2%) seems great; however, no change exists in the absolute number of band neutrophils in the blood (i.e., 200 bands/μl of blood). The relative percentage of band neutrophils between the second and third bars seems identical. However, a normal number of band neutrophils exist in the dog with 200 bands/μl of blood, but a true increase (left shift) in band neutrophils occurs in the dog with 1200 bands/μl in the third bar. *WBC,* White blood cell.

For initial leukogram evaluation, absolute cell counts for each leukocyte type should be interpreted individually. Subsequent changes in the leukogram can be summarized by a few hematologic terms indicating an increase or decrease in a given leukocyte type. For example, leukocytosis with mature neutrophilia, lymphopenia, and monocytosis indicates succinctly that the complete blood count (CBC) had an increased WBC count, an increase in the number of mature segs, and no increase in the number of band

neutrophils; that the lymphocyte count was decreased; and that the monocyte count was increased, respectively.

> NOTE: Absolute cell numbers rather than percentages are used to evaluate the leukogram.

Blood Leukocyte Production, Circulation, and Emigration

To interpret the concentration of leukocytes in the blood, one must consider rate of leukocyte production in bone marrow and release into blood, distribution and circulating half-life of leukocytes, and rate of emigration of leukocytes from blood into tissues.

Leukocyte Production

Granulocytes (i.e., neutrophils, eosinophils, basophils) and monocytes are produced in the bone marrow. Although the bone marrow produces some lymphocytes, most are derived primarily from the peripheral lymphoid tissues (i.e., thymus, lymph nodes, spleen, tonsil, bronchial-associated lymphoid tissue, gut-associated lymphoid tissue). Leukocytes develop in the bone marrow from pluripotent and committed stem cells influenced by interleukins and colony-stimulating factors (Raskin, 1996). Pluripotent stem cells provide a reserve compartment for hematopoietic cell (i.e., leukocyte, erythrocyte, platelet) production if bone marrow has been severely damaged by toxins, drugs, infectious agents, or radiation (Figure 4-2). Pluripotent stem cells are found in both bone marrow and blood. They may affect repopulation of the bone marrow after a severe insult, provided proper stromal cells and growth factors are present. The number of pluripotent stem cells decreases with age, so younger animals generally have a better chance of restoring bone marrow function than do aged adults.

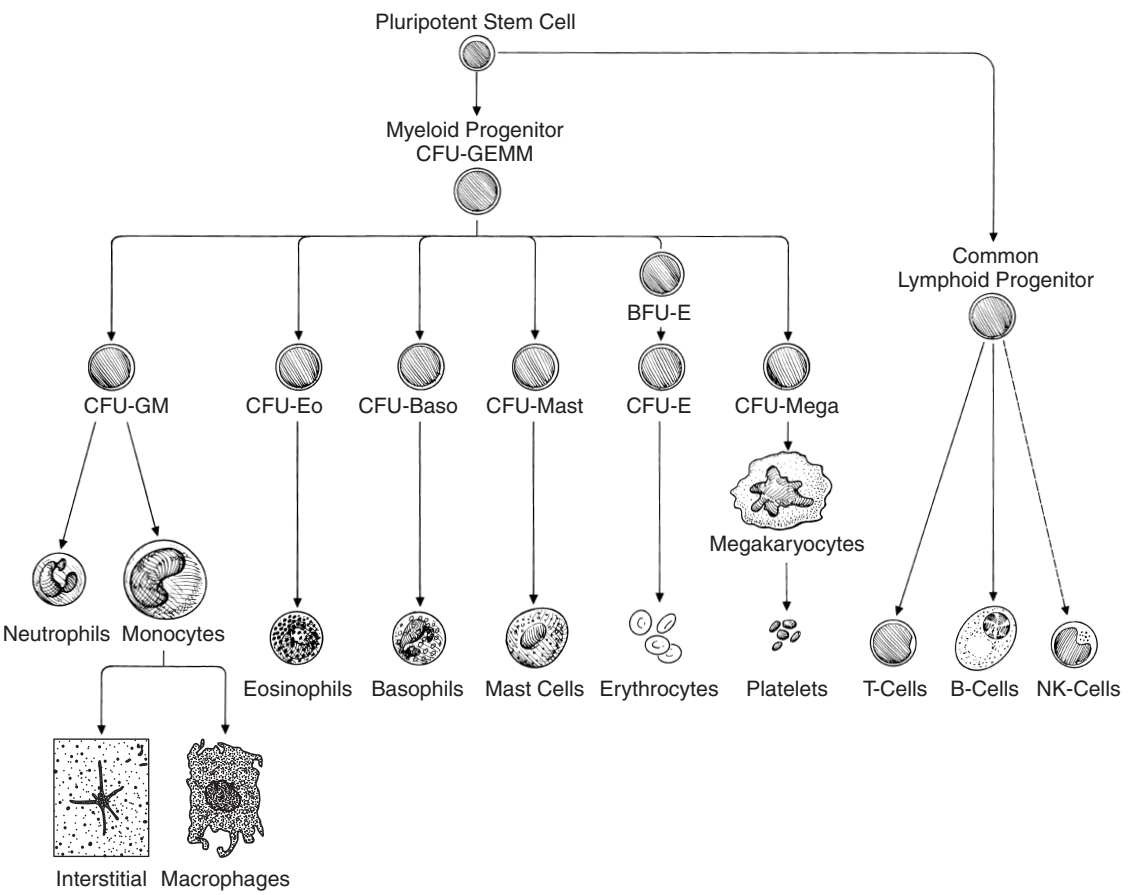

FIGURE 4-2. Differentiation of the bone marrow pluripotent stem cells. *Dotted lines* reflect uncertainty in derivation. *CFU*, Colony forming unit; *GEMM*, granulocyte-erythroid-monocyte-megakaryocyte; *GM*, granulocyte-macrophage; *BFU*, burst-forming unit; *E*, erythroid; *Mega*, megakaryocytic; *Eo*, eosinophil; *Baso*, basophil; *DC*, dendritic cell; *NK*, natural killer.

The following discussion of bone marrow leukocyte production emphasizes production of neutrophils. Cellular "pools" are used to conceptualize the "location" of neutrophils within the bone marrow and blood and to simplify interpretation of bone marrow and CBC data (Figure 4-3). Bone marrow is divided into two pools: The first, the mitotic pool of myeloblasts, promyelocytes, and myelocytes, provides a steady supply of neutrophils to meet tissue demand for these cells. The second pool is maturation and storage, which consists of metamyelocytes, bands, and segmenters that lack mitotic ability. These cells undergo progressive maturation and provide a reserve of segs to meet increased tissue demands for neutrophils until the mitotic pool increases neutrophil production. The maturation and storage functions are combined in Figure 4-3. Neutrophil release from the bone marrow into the blood is age ordered according to maturity (segs, bands, metamyelocytes, myelocytes, and promyelocytes, in that order). As bone marrow stores of segs are depleted, nonsegmented neutrophils (nonsegs) (e.g., bands, metamyelocytes, younger neutrophils) are released into the blood, and a left shift occurs.

The maturation and storage pool constitutes 80% of the myeloid cell population, whereas the mitotic pool usually accounts for 20% of the myeloid series. In contrast with neutrophils, promonocytes and monocytes are released into blood at a relatively young age. This lack of monocyte maturation and storage in the bone marrow explains why monocytes are observed infrequently in most bone marrow aspirates, unless severe neutropenia is present.

Leukocyte production within the bone marrow can be evaluated by bone marrow aspiration for cytology and core biopsy for histopathology. Romanowsky-stained aspirates of marrow allow qualitative and quantitative observations on leukocyte morphology and maturation. Core biopsy provides the best estimation of bone marrow cellularity and detects stromal reactions (e.g., myelofibrosis, granulomatous osteomyelitis). If the bone marrow fails to produce sufficient leukocytes, erythrocytes, or platelets, then leukopenia, anemia, or thrombocytopenia (or a combination thereof) results. In contrast, accelerated production of these cellular elements produces leukocytosis, polycythemia, thrombocytosis, or a combination thereof.

> NOTE: The production of segs from bone marrow myeloblast takes approximately 6 days in the dog and cat but shifts from the marginal pool, or increased release from the storage pool can influence the number of blood neutrophils within minutes to hours.

Neutrophil Circulation

When granulocytes and monocytes are released into the blood, they distribute between circulating and marginal cell pools, circulate for a brief period of time, and emigrate from blood vessels into tissues. In the blood, the total blood neutrophil pool (TBNP) is subdivided into circulating and marginal cell pools. Neutrophils (and other leukocytes) in the total leukocyte count are collected from the circulating pool, which encompasses the mainstream or central axial flow of blood within vessels. The marginal neutrophil pool is a "hidden" population associated with the

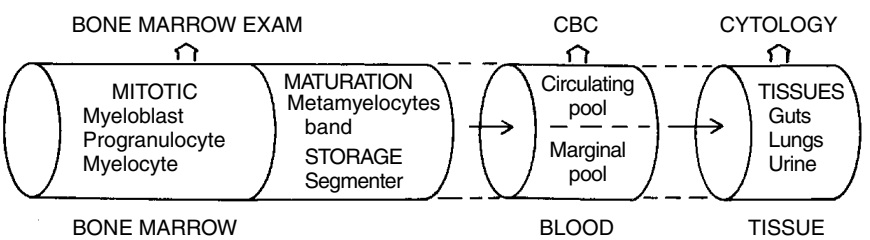

FIGURE 4-3. Neutrophil compartments in the body. Neutrophils in various areas of the body are grouped into pools for evaluation. The bone marrow cells are divided into the mitotic, maturation, and storage pools (see text). Neutrophils in blood are either in the circulating pool, which is sampled by a complete blood count (CBC), or the marginal pool, which is hidden from sampling via the CBC. Neutrophils move in one direction into the tissues, where they can be evaluated by cytology or histopathology. *(Modified from Boggs DR, Winkelstein A:* White cell manual*, ed 3, Philadelphia, 1975, FA Davis.)*

TABLE 4-1. Total Blood Neutrophil Pool, Circulating Neutrophil Pool, and Marginal Neutrophil Pool in Dogs and Cats

	DOG	CAT
TBNP $\times 10^8$/kg	10.2	28.9
CNP $\times 10^8$/kg	5.4	7.8
MNP $\times 10^8$/kg	4.8	21.0

Total blood neutrophil pool *(TBNP)* in cats is larger than in dogs because of a very large marginal neutrophil pool. The relatively large feline marginal neutrophil pool *(MNP)*, compared with that of the dog, allows a larger potential shift of neutrophils into the circulating neutrophil pool *(CNP)* with more dramatic leukocytosis during fear, excitement, or strenuous exercise.

endothelial lining of capillaries, especially the lungs and spleen. As cells continually shift between these pools, the leukocyte count changes. In dogs, circulating and marginal pools are about equal. In cats, the marginal pool is two to three times the size of the circulating pool (Table 4-1). Therefore if neutrophils are mobilized from the marginal pool to the circulating pool in response to fear, excitement, or strenuous exercise, the neutrophil count might double in dogs and triple in cats.

Neutrophil Emigration into Tissues

Neutrophils normally spend about 10 hours in the vascular system before emigrating from the blood vessels into the tissues. Emigration is a random (i.e., nonage ordered) and unidirectional event (these cells do not return to the circulation). In health, neutrophils primarily migrate into the respiratory, digestive, and urinary tracts at a low rate in response to bacteria and other stimuli. Although neutrophils may be visible in respiratory cytologic samples and urine sediment, they are quickly lysed in the septic environment of the lumen of the bowel. In disease, the circulating half-life of neutrophils may be shortened considerably, accompanied by increased cell migration into tissues. In inflammation, excessive tissue neutrophils may be visible as exudate or pus. In diseases such as enteritis, tissue neutrophils may be hidden from cytologic or gross observation; however, increased tissue demand for neutrophils usually is reflected in the leukogram.

The CBC allows quantitative and qualitative observations about leukocytes freely circulating in peripheral blood. The leukogram represents balance of leukocyte production in bone marrow, distribution in the vascular system, and emigration from blood vessels into tissues. One uses cytologic preparations such as smears of exudate; bone marrow, lymph node, and other tissue aspirates; and urine sediment to evaluate leukocytes and infectious agents in tissues.

LEUKOCYTOSIS AND NEUTROPHILIA

Leukocytosis is usually synonymous with neutrophilia. For example, in 232 CBC with a leukocytosis of greater than 17,000 WBCs/μl, 226 (97.4%) had neutrophilia.

Differential Diagnosis of Neutrophilic Leukocytosis

The differential diagnosis of neutrophilic leukocytosis includes inflammation, stress and corticosteroids, exercise and epinephrine, or leukemia (discussed later). An algorithm (Figure 4-4) differentiates the initial three possibilities. Inflammation is most specifically

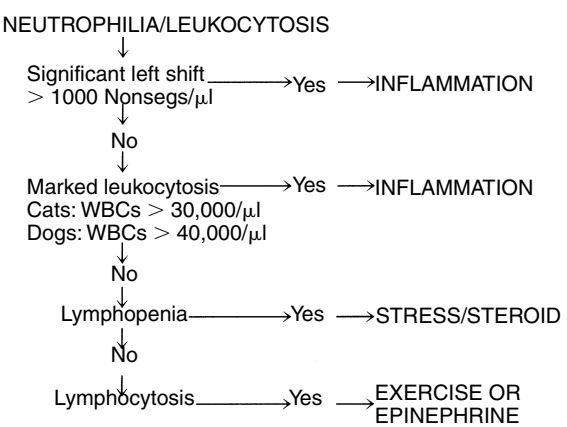

FIGURE 4-4. Evaluation of leukocytosis and neutrophilia. The common causes of neutrophilia and subsequent leukocytosis usually may be differentiated based on the immaturity of the neutrophils, the magnitude of the neutrophilia in the absence of leukemia, and the tendency of the lymphocytes to increase or decrease. Granulocytic leukemia is rare and not considered here. When the laboratory finding is present *(Yes)*, the conclusion to the right is made. When the laboratory finding is absent *(No)*, one moves down to the next differentiating feature. Inflammation may be responsible for total white blood cell *(WBC)* counts of less than 30,000 to 40,000 leukocytes/μl that are associated with lymphopenia because of concurrent stress. Some leukograms lack features to clearly indicate the causes of disease.

identified by the presence of a left shift (an absolute increase in nonsegs). Inflammation may also be suggested by the magnitude of leukocytosis (if it exceeds that expected with corticosteroid or epinephrine-associated conditions). When mild neutrophilia is present without a left shift, the specific cause of leukocytosis may be unclear. In such instances, the absolute lymphocyte count may be useful. Lymphopenia usually indicates an endogenous stress-associated or exogenous glucocorticoid effect, whereas transient lymphocytosis implies an epinephrine or exercise-associated effect. Processes may often occur concurrently, such as both inflammation and stress or corticosteroid treatment.

NOTE: Leukocytosis has several causes: inflammation, glucocorticoid response from stress or treatment, epinephrine response from fear or exercise, and leukemia.

Inflammation

Inflammation is a common and important laboratory diagnosis. Acute inflammation usually causes neutrophilia and is the major consideration for neutrophilic leukocytosis. Neutrophils predominate in suppurative or exudative diseases but may be admixed with other inflammatory cells (e.g., lymphocytes, plasma cells, monocytes and macrophages, eosinophils). Although bacterial infection (i.e., sepsis) commonly causes neutrophilic exudation, purulent to pyogranulomatous inflammation also may occur with certain mycotic, protozoal, and viral infections (especially feline infectious peritonitis). Inflammation also may occur from nonseptic processes such as necrosis (e.g., pancreatitis, pansteatitis), chemical exposure (e.g., turpentine is an experimental method of abscess formation), immune-mediated diseases (e.g., systemic lupus erythematosus, immune-mediated hemolytic anemia [IMHA]), and toxins (e.g., endotoxin, snakebite). Neoplasms may cause inflammation in five ways: (1) predisposing the patient to bacterial infection, (2) damaging normal tissue, (3) outgrowing or damaging the blood supply with subsequent necrosis, (4) ulceration, or (5) producing a paraneoplastic effect wherein tumor products stimulate the bone marrow to produce neutrophils.

NOTE: Inflammation is recognized by a left shift in neutrophils or marked nonneoplastic leukocytosis.

Left Shift

A significant left shift is denoted by the finding of greater than 1000 nonsegs/µl in the presence of a normal neutrophil count or neutrophilia and is diagnostic for the presence of inflammatory disease (see Figure 4-4). Milder left shifts (i.e., 300 to 1000 nonsegs/µl) occur in hemorrhagic, chronic, or granulomatous diseases. Absolute number of nonsegs and their state of immaturity indicate the severity of the left shift. Immature nonsegs observed in blood include bands (stabs), metamyelocytes (juveniles), myelocytes, and promyelocytes (see Color Plate 2F). Bands usually constitute most of the left shift because release of neutrophils from bone marrow is an age-ordered process. Neutrophils younger than bands indicate an increasingly severe left shift associated with increasingly intense inflammation. Classification of immature neutrophils on blood smears is very subjective; therefore one should not overinterpret small changes from reference values or from day to day. If several myelocytes and metamyelocytes are found, one should report the number of metamyelocytes, myelocytes, and promyelocytes (not simply group them under the heading of *nonsegs*) to indicate severity of the left shift. Detectable numbers of blast cells (i.e., myeloblasts) or irregular maturation patterns may suggest granulocytic leukemia (discussed later).

NOTE: A left shift is an absolute and clinically significant increase in immature neutrophils and is the most specific indicator of inflammation.

Leukogram Changes in Inflammation

Figure 4-5 gives a basic pattern of leukogram changes to expect during an inflammatory disease. Individual animals will vary from this pattern in response to drug administration or to variations in disease intensity. The greatest left shift would be expected in early stages of the disease process. As the preexisting bone marrow maturation and storage pool is depleted of segs, then neutrophil bands

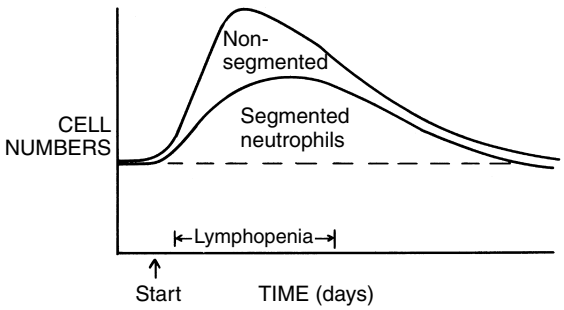

FIGURE 4-5. Expected leukocyte changes with resolving inflammation. The greatest leukocytosis and left shift are expected early in acute inflammation. This period is also accompanied by the lymphopenia of stress. During later phases of inflammation, a more mature form of neutrophilia is expected, because bone marrow hyperplasia and marrow production of neutrophils should be adequate to allow maturation of neutrophils before release into blood. Tissue demand for neutrophils also tends to decrease during recovery.

and metamyelocytes are released. Over ensuing days, myeloid hyperplasia within the bone marrow expands neutrophil production. If neutrophil production and maturation time are sufficient, mainly mature cells are released into the blood and the severity of the left shift should diminish. If tissue inflammation stabilizes at a persistent low-to-moderate level, the bone marrow should reach a production rate sufficient for most neutrophils to mature before release. Thus chronic inflammation may be characterized by little to no left shift and minimal to absent leukocytosis. This form of chronic inflammation is the hardest to identify by CBC data alone. Cytology of the affected tissue may detect low-grade purulent inflammation or exudation.

NOTE: The second most common diagnosis from a leukogram is that inflammation is present in the animal. The leukogram also characterizes the type and severity of the inflammatory disease.

A normal leukogram appearance need not exclude inflammatory diseases, especially if inflammation is mild, chronic, or only involves a surface (e.g., cystitis). No test is 100% sensitive. Increased rouleaux (see Chapter 2) in canine blood smears or fever suggests inflammation. Increased rouleaux formation usually is associated with production of acute phase proteins such as fibrinogen.

Bone Marrow Response During Inflammation

Myeloid hyperplasia of the bone marrow is expected with inflammation of greater than 2 to 3 days' duration. Leukocytosis and left shift are usually present, so a bone marrow sampling is not needed to prove the marrow is active. Myeloid hyperplasia in bone marrow aspirates usually is identified while one is investigating another hematologic problem, such as nonregenerative anemia, thrombocytopenia, or suspected leukemia (see Chapter 2). In these situations, concurrent inflammation and infection, necrosis, or generalized bone marrow stimulation may cause myeloid hyperplasia.

Prognosis

Magnitude of the neutrophilic leukocytosis or neutropenia during inflammation reflects the balance of bone marrow production and tissue demand for these cells. If the bone marrow is responding typically to an inflammatory process with a mild-to-moderate left shift, prognosis is relatively good. Leukocytosis in dogs is usually less than 40,000 WBCs/μl. In 182 canine CBC with leukocytosis, 151 CBC (83%) had 17,500 to 39,990 WBCs/μl. Leukocytosis in this range suggests a favorable prognosis. Only 5% had marked to extreme leukocytosis of 61,050 to 127,500 cells/μl. Leukemoid reactions in this range indicate a poor prognosis. The magnitude of feline leukocytosis is usually less than in dogs (i.e., 70% of cases are < 30,000 WBCs/μl). Obviously, other factors not derived from the CBC data, such as the cause and site of inflammation, also affect the prognosis.

Criteria suggesting a poor prognosis are summarized in Table 4-2. A degenerative left shift, leukopenia and neutropenia, leukemoid reaction, or a combination thereof is an atypical, unexpected response to inflammatory disease indicating severe disease, inadequate bone marrow production, problems interfering with an effective response, or a combination of these. *Lymphopenia* indicates stress. Severe or persistent lymphopenia indicates severe or persistent stress. The most common, current definition of a degenerative left shift is that nonsegs exceed the number of segmenters, regardless of total leukocyte count. Finding *more nonsegs than circulating* segs indicates that the bone marrow cannot produce neutrophils at a rate sufficient for

TABLE 4-2. Leukogram Findings Considered Poor Prognostic Indicators

FINDING	REASON FOR POOR PROGNOSIS
Degenerative left shift	Tissue demand exceeds bone marrow's production of neutrophils or causes inadequate time for maturation of neutrophils
Leukopenia	Tissue demand exceeds bone marrow's production of neutrophils
Leukemoid reaction	Even excessive neutrophils cannot correct the cause
Significant toxic neutrophils	Moderate-to-many, moderately to severely toxic neutrophils indicate toxemia, often gram-negative sepsis
Severe or persistent lymphopenia	Indicates severe stress or lack of relief from stress

them to mature properly. Either cell production is decreased, tissue demand for neutrophils has escalated dramatically, or both; a guarded prognosis is indicated. Severity of change and trend over daily hemograms are important in assessing prognosis. A degenerative left shift, severe neutropenia and leukopenia, lymphopenia, and *marked toxic change* in most neutrophils suggest a poor prognosis and usually gram-negative sepsis.

In *severe leukopenia* (e.g., < 1000 WBCs/µl), an increase in the absolute number of immature neutrophils may not be observed. Both leukopenia and neutropenia are unfavorable prognostic signs. These findings suggest that bone marrow is incapable of producing sufficient numbers of neutrophils, that tissue consumption of neutrophils is overwhelming, or both. Neutropenia, either primary or secondary, severely predisposes the patient to infection and septicemia.

A *leukemoid reaction* is a marked leukocytosis (≥ 50,000 to 100,000 WBCs/µl) as the result of inflammation. The magnitude of leukocytosis is leukemia-like. A leukemoid reaction indicates a poor prognosis, because despite abundant (and actually excessive) neutrophils, the inflammatory disease is not being corrected. Causes of leukemoid reactions include severe localized infections (e.g., pyometra, abscess), IMHA, paraneoplastic syndromes with bone marrow stimulation (e.g., metastatic fibrosarcoma, renal carcinoma, rectal adenoma), rare parasitism (e.g., *Hepatozoon canis* infection), and neutrophil functional defects (canine leukocyte adhesion protein deficiency [CLAD] of Irish setters) (Latimer, 1995; Latimer, Campagnoli, and Danilenko, 2000). With pyometra and some walled off abscesses, there is an anatomic problem. Infection and pus cannot drain from the body, and antibiotics may not penetrate the lesion. With CLAD, dysfunctional neutrophils are incapable of correcting common infections even in high numbers. The leukemoid reaction in IMHA seems an exception, but acute destruction and phagocytosis of erythrocytes with hypoxic tissue damage is a strong stimulus for an inflammatory reaction.

Differentiation of a leukemoid reaction from chronic granulocytic leukemia is difficult. The presence of one of the typical inflammatory diseases associated with a leukemoid reaction should be documented initially. Leukocytosis should resolve if the cause of inflammatory disease is discerned and treated appropriately. A guarded prognosis is indicated until the possibility of granulocytic leukemia is excluded and the leukocytosis begins to resolve after treatment. Leukemoid reactions lack the blast cells or atypia that occurs in acute granulocytic leukemia (see the discussion of myeloproliferative disease later in this chapter). Left shifts in leukemoid reactions usually involve bands and metamyelocytes (perhaps even with myelocytes and promyelocytes). Signs of toxicity are common.

NOTE: A guarded to poor prognosis is indicated by the presence of a degenerative left shift, leukopenia, leukemoid reaction, or moderate-to-marked neutrophil toxicity.

Leukoerythroblastic Reaction

The leukoerythroblastic reaction is uncommon but mimics erythroleukemia. A leukoerythroblastic reaction occurs when immature erythroid and myeloid cells are released into circulation in various diseases (e.g., bone marrow necrosis, severe hemolytic or blood loss anemia, hemangiosarcoma, extramedullary hematopoiesis, bone marrow metastatic disease) that disrupt the bone marrow-blood barrier, resulting in premature hematopoietic cell release into the blood.

Toxic Neutrophils

Toxic neutrophils (see Chapter 2) indicate toxemia and commonly accompany severe inflammation and infection. Bacterial toxins cause the most severe toxic changes in neutrophils; however, nonbacterial toxins (e.g., chemotherapeutic agents) also may be responsible.

TABLE 4-3. Leukocyte Changes in a Dog Treated with Dexamethasone

	FRIDAY	SATURDAY	MONDAY	TUESDAY	WEDNESDAY
WBCs/μl	12,200	22,200	19,600	31,100	29,300
Segs/μl	9525	18,648	10,976	26,433	25,491
Bands/μl	0	0	196	0	0
Lymphocytes/μl	1905	1998	5096	1866	879
Monocytes/μl	635	1554	2156	2799	3132
Eosinophils/μl	635	0	1176	0	0

Hematologic data are from an apparently normal dog treated daily with dexamethasone (except on Sunday) to illustrate the corticosteroid and stress response. Data for Friday, the first day before treatment, should be used for baseline (reference) values. Exceptions to the classic pattern occurred every day except Wednesday.
WBCs, White blood cells.

Classification of toxemia is based on the number of neutrophils affected (percentage; or few, moderate, many) and severity of morphologic change (1+ to 4+ toxic change). A few (1+) toxic neutrophils are of minimal importance, but moderate-to-many (2+ to 4+) toxic neutrophils are a poor prognostic sign (see Color Plate 2F). For example, severely toxic neutrophils in a young dog with bloody diarrhea suggest parvovirus infection. Abundant bacterial toxins absorbed from the damaged bowel promote toxic neutrophil changes. Toxic changes in neutrophils sometimes are the only indicator of disease (the rest of the leukogram may appear normal) and are one reason why one should always look at the blood smear.

Stress and Corticosteroid Response

Stress and corticosteroid administration are common causes of neutrophilia. The classic, acute leukogram pattern is moderate leukocytosis with mature neutrophilia, lymphopenia, and eosinopenia. In dogs, mild-to-moderate monocytosis also may occur (e.g., 2500/μl). Leukograms that indicate changes secondary to endogenous or exogenous steroids are common. Leukocytosis from corticosteroid treatment in dogs may reach 30,000 to 40,000 cells/μl, with a predominance of neutrophils. The typical response is 15,000 to 25,000 WBCs/μl (Duncan, Prasse, and Mahaffey, 1994).

Neutrophilia develops in 4 to 12 hours and returns to baseline values in less than 24 hours. In cats, leukocytosis usually is a little weaker (e.g., 22,000 WBCs/μl with 18,000 neutrophils/μl), and monocytosis is not present. The chronic hematologic pattern from hyperadrenocorticism and long-term corticosteroid treatment varies (including normal), but expected changes include lymphopenia, eosinopenia, and a normal neutrophil count (Latimer, 1995; Moore, Mahaffey, and Hoeing, 1992). Coexisting processes may have opposing stimuli (e.g., a dog may have a disease stimulating eosinophilia and be stressed or treated with corticosteroids and thus have a variable eosinophil count).

> NOTE: Lymphopenia of stress and corticosteroid treatment is the most common alteration in the leukogram of dogs and cats.

The effects of corticosteroid treatment on the canine leukocyte response are better understood by considering Tables 4-3 and 4-4. After corticosteroid exposure, the TBNP expands because of increased release of neutrophils from the bone marrow into the blood and decreased emigration of neutrophils from the blood into the tissues (see Table 4-4). In addition, neutrophils are shifted from the marginal pool (where they cannot be counted) to the circulating pool (where they are quantitated by the WBC count). A left shift is not expected with stress or corticosteroid treatment. Nuclear hypersegmentation of neutrophils (called a *right shift*) is more likely because corticosteroids decrease emigration of and prolong the circulating half-life of neutrophils in the blood (see Table 4-4). As neutrophils age, progressive nuclear hypersegmentation or lobulation develops. Hypersegmented neutrophils have five or more nuclear lobes. Corticosteroids also increase the bone marrow release rate of neutrophils, which is a major cause of the leukocytosis. However, this effect is usually too mild to stimulate release of bands and metamyelocytes in the presence of a normal bone marrow storage pool of neutrophils. A left shift indicates a depleted bone marrow

TABLE 4-4. Effects of Cortisone on Canine Granulocytes

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storage pool of neutrophils and significant tissue demand for neutrophils secondary to concurrent inflammation.

All expected corticosteroid-induced changes may not be present in every leukogram. A "best fit" approach is used to classify leukograms. For example, Table 4-3 presents hematologic data from a healthy dog treated repeatedly with dexamethasone. One day after initial treatment (Saturday), five of the expected steroid and stress features occurred (i.e., leukocytosis, neutrophilia, no left shift, eosinopenia, monocytosis). Lymphopenia was not present, although usually this is the most consistent change. On day 3 (Monday), lymphocytosis, monocytosis, and eosinophilia best resemble physiologic leukocytosis. Only on day 5 (Wednesday) is the classic corticosteroid or stress pattern observed. Some of the variation may be the result of the time between treatment with dexamethasone and collecting the blood sample. Maximal leukocyte changes occur at 4 to 12 hours and may be normalized by 24 hours.

NOTE: Corticosteroid treatment will cause a neutrophilia, lymphopenia, eosinopenia, and (in dogs) monocytosis. The magnitude of changes will vary during a 24-hour period after treatment.

Exercise and Epinephrine Response

Transient physiologic leukocytosis is noted mainly in young, healthy cats during epinephrine release from fear or after strenuous exercise (e.g., struggling during venipuncture). The TBNP remains unchanged, but a shift of cells occurs from the marginal to the circulating neutrophil pool. Subsequently, neutrophilic leukocytosis is detected. No increased release of neutrophils from the bone marrow, nor decreased emigration of neutrophils from the capillary beds is seen. Physiologic leukocytosis in cats is greater in magnitude than in dogs because cats have a larger marginal neutrophil pool (three neutrophils in the marginal pool for every neutrophil in the circulating pool; see Table 4-1). These pool shifts in cats are significant; the WBC count often reaches 20,000/µl, and neutrophilia may be overshadowed by lymphocytosis (6000 to 15,000/µl) (Duncan, Prasse, and Mahaffey, 1994). Dogs have such weak physiologic leukocytosis that it is not recognized clinically because values likely remain within reference ranges. It is best seen in research dogs that are routinely bled and have a record of consistent hemograms and then are suddenly exercised or frightened so that the mild increase in WBCs, neutrophils, lymphocytes, and packed cell volume (PCV) can be detected.

NOTE: Physiologic release of epinephrine (fear or vigorous activity) causes an immediate, mild-to-moderate increase in neutrophils and lymphocytes that should subside gradually over 30 to 60 minutes.

LEUKOPENIA AND NEUTROPENIA

Leukopenia and neutropenia occur infrequently in dogs and cats and constitute a poor prognostic sign. The two most likely causes of neutropenia are (1) excessive tissue consumption of neutrophils during severe inflammation and (2) disrupted bone marrow production (Table 4-5). (See also the discussion of bone marrow in Chapter 2

TABLE 4-5. Major Causes of Neutropenia

	ANIMALS AFFECTED
Consumption of Neutrophils	
Overwhelming sepsis/endotoxemia (important)	Dogs/cats
Parvovirus enteritis (important)	Dogs/cats
Salmonellosis	Dogs/cats
Immune-mediated destruction (rare)	Dogs
Bone Marrow Suppression	
Feline leukemia virus (FeLV) (important)	Cats
Feline immunodeficiency virus (FIV)	Cats
Parvovirus (important)	Dogs/cats
Ehrlichiosis	Dogs
Bone marrow toxicity	Dogs/cats
Estrogen (endogenous/exogenous)	Dogs
Phenylbutazone*	Dogs/cats
Cancer chemotherapy	Dogs/cats
Irradiation	Dogs/cats
Leukemia (important)	Dogs/cats
Myelophthisis/myelonecrosis	Dogs/cats
Immune-mediated destruction of neutrophil precursors (rare)	Dogs/cats

*Incomplete list of other drugs is in text.
Courtesy of Dr. M.D. Willard.

and the section on myeloid hypoplasia, discussed later.) A third cause of neutropenia, only documented in experimental settings, is a temporary shift of neutrophils from the circulating to the marginal pool where they cannot be counted. Endotoxin can cause this change. This transient form of neutropenia is actually "pseudoneutropenia," because the TBNP is unchanged. Rarely, immune-mediated or "steroid-responsive" neutropenia occurs with lupus or some drugs.

During excessive tissue utilization of neutrophils, neutropenia is severe; this stimulates release of very immature neutrophils, even myelocytes and promyelocytes, from the bone marrow. A clinically important left shift is present when 10% or more of the total neutrophil population are bands or younger forms of neutrophils. The left shift is degenerative when bands and younger forms of neutrophils outnumber the segmenters. An inflammatory process involving a large surface area, such as septic peritonitis, enteritis, or septicemia, tend to cause severe neutropenia and leukopenia. Gram-negative bacterial infections are frequently associated with consumptive neutropenia. When a severe left shift, marked toxic change, and developing leukopenia occur, gram-negative sepsis should be suspected. In contrast, localized infections (abscess or pyometra) with pyogenic bacteria usually cause leukocytosis.

Hemograms may differentiate leukopenia of overwhelming infection from that of primary bone marrow disease. Neutrophils have a short life span in blood, and their numbers should decrease before anemia or thrombocytopenia develop. This is usually the pattern in severe inflammation or overwhelming infection. Pancytopenia or bicytopenia suggests primary bone marrow disease, but neutropenia of overwhelming inflammation may coexist with anemia or thrombocytopenia of other causes (e.g., hemorrhage, disseminated intravascular coagulation, or both). When the clinician is unsure of the problem, bone marrow aspiration and core biopsy are indicated to evaluate hematopoiesis. Time course of the disease process may assist in differentiating these two causes of neutropenia. Primary bone marrow disease may develop insidiously, whereas consumptive neutropenia develops rapidly. A left shift, toxic changes in neutrophils, and rouleaux formation suggest neutropenia as the result of severe inflammation or infection.

Severe, primary neutropenia can predispose to septicemia. Neutropenia and leukopenia occur with myelotoxicity during cancer chemotherapy. Neutrophil counts are often lowest 5 to 7 days after initiation of treatment. Guidelines may predict sepsis (Couto, 1985). Neutrophil counts of less than 2000 cells/µl require monitoring the patient for sepsis. Sepsis (probably from enteric bacteria) is presumed to be present if the patient has less than 500 neutrophils/µl and is febrile. Furthermore, chemotherapy with myelosuppressive agents should be discontinued if the neutrophil count is less than 2500 cells/µl or the platelet count is less than 50,000/µl.

Bone Marrow Diseases Causing Neutropenia

Persistent, unexplained neutropenia is an indication for bone marrow aspiration biopsy, core biopsy, or both. Possible causes of decreased myelopoiesis include decreased production of neutrophils (i.e., myeloid hypoplasia, ineffective granulopoiesis, bone marrow necrosis) or bone marrow lesions (i.e., myelofibrosis, disseminated granulomatous infection, leukemia) that displace normal hematopoietic tissue (see Chapter 2). A cause (e.g., estrogen or phenylbutazone toxicosis) is infrequently discovered by examination of bone marrow; however, one may determine whether myeloid hypoplasia or myelophthisic disease is responsible.

Ineffective granulopoiesis is suggested when leukopenia is accompanied by normal to increased numbers of developing myeloid cells in the bone marrow (in the absence of overwhelming infection). Cells are destroyed in the bone marrow ("recycled") before maturation and so are not released into the blood. This situation occurs to a small degree in healthy dogs (i.e., the myelocyte sink) but is exaggerated in diseases such as canine parvovirus infection and feline leukemia virus (FeLV) infection. Approximately 50% of FeLV-positive, neutropenic cats have marked granulocytic hyperplasia with a shift to immaturity, whereas 50% have myeloid hypoplasia suggesting viral destruction of hematopoietic tissue. Persistent neutropenia despite myeloid hyperplasia in FeLV-infected cats indicates ineffective granulopoiesis, provided increased tissue consumption of neutrophils is not responsible for the neutropenia. Ineffective granulopoiesis may also be a drug idiosyncratic reaction (e.g., phenobarbital).

Leukopenia and neutropenia may also be the result of damage or depletion of myeloid cells (i.e., myeloid hypoplasia) (see Chapter 2). Causes of myeloid hypoplasia include parvovirus infection, endogenous and exogenous estrogen toxicosis in dogs, *Ehrlichia canis* infection, cancer chemotherapy, irradiation, and idiosyncratic reactions to drugs such as phenylbutazone, trimethoprim-sulfadiazine, or chloramphenicol (see Chapter 3). Although immune-mediated neutropenias have not been well documented in dogs and cats, steroid-responsive neutropenias have occurred in both species. Artifactual neutropenia may occur from leukocyte aggregation after exposure to ethylenediaminetetraacetic acid (EDTA) anticoagulant or by obtaining diluted blood specimens from intravenous fluid administration lines.

Parvovirus Infection

Parvovirus infection may be associated with diarrhea, vomiting, and leukogram abnormalities that may include leukopenia, neutropenia with severe toxic changes, and lymphopenia. *Parvovirus* infects and destroys rapidly dividing cells (e.g., intestinal crypt epithelium, lymphoid tissue, hematopoietic cells). This tissue predilection may cause enteritis (with or without bloody diarrhea) or leukopenia. Massive neutrophil exudation into the damaged gut contributes to leukopenia. Leukopenia is transient and usually occurs early in canine parvovirus disease, so it may be missed without multiple CBC. Mast cells from the inflamed gut may be detected in blood smears. Neutrophilic leukocytosis is expected with recovery. During periods of intense granulopoiesis, a leukemoid reaction plus some disturbance of normal cell maturation may occur. With complete clinical recovery, baseline leukogram values are regained. Antemortem diagnosis of parvovirus enteritis is accomplished most easily by detection of virus in feces (see Chapter 9). Panleukopenia in cats has a similar hematologic response.

Feline Leukemia Virus Infection

FeLV infection causes various disorders (see Chapter 15) such as cytopenias, including neutropenia (Brown and Rogers, 2001). FeLV viremia is diagnosed mainly by enzyme-linked immunosorbent assay (ELISA) procedures and indirect fluorescent antibody (IFA) examination of blood smears or buffy coat smears. If ELISA testing for FeLV group-specific antigen is negative but FeLV infection is still suspected, bone marrow aspirates should be examined via IFA to exclude the possibility of sequestered virus.

Feline Immunodeficiency Virus Infection

Feline immunodeficiency virus (FIV) infection may be associated with cytopenias (i.e., neutropenia, anemia, thrombocytopenia) (Shelton, Linenberger, Abkowitz, 1991). Many diseases have been associated with FIV, including various infections, malignancies, lymphadenopathy, colitis, and central nervous system disorders. Diagnosis of FIV involves ELISA and Western blot procedures available at various laboratories (see Chapter 15).

Cyclic Hematopoiesis

Cyclic hematopoiesis (i.e., gray collie syndrome, cyclic neutropenia) is an autosomal recessive disease characterized primarily by cyclic neutropenia with 11- to 12-day cycles described originally in silver-gray collie pups. Neutropenia as severe as 0 to 400/µl predisposes affected collies to life-threatening bacterial infections. A stem cell defect causes cyclic decreases in production of platelets, other granulocytes and monocytes, and erythrocytes (i.e., reticulocytes). Because of a longer half-life of platelets and erythrocytes,

numeric change in these elements is less noticeable than for neutrophils. Cyclic hematopoiesis, often with a more irregular periodicity, also has been observed in other breeds of dogs, in rare cats with FeLV infection (Swenson et al, 1987), and after cyclophosphamide treatment of some dogs. Oscillations of neutrophils, other leukocytes, reticulocytes, and platelets occur at 8- to 29-day intervals.

Hemograms obtained at 2- to 3-day intervals should document cyclic neutropenia, but daily CBC may be required to document cycling of other cells and platelets.

MONOCYTOSIS AND MONOCYTOPENIA

Monocytosis occurs in about 30% of hospitalized dogs and 11% of cats. Blood monocytes replenish macrophages in tissues. Tissue macrophages include Kupffer's cells in hepatic sinusoids, pulmonary alveolar macrophages, dendritic cells in lymph nodes, and microglial cells in the brain (in addition to histiocytes and multinucleate giant cells occurring in response to inflammation). Maturation from blood monocytes to tissue macrophages is accompanied by changes in cellular morphology, metabolic activity, enzyme content, and synthesis of biologically active proteins.

Macrophages remove necrotic debris, kill fungi and some parasites, inactivate viruses, respond to foreign bodies, phagocytose senescent and abnormal red blood cells (RBCs), and destroy neoplastic cells. Monocytosis is expected in inflammatory diseases with a high need for macrophages. In IMHA, for example, RBCs coated with antibody or complement are being destroyed continuously. Necrotic cell debris also must be removed to allow tissue regeneration and healing. Although macrophages are a "late" component of most inflammatory processes, monocytosis may occur in both acute and chronic disease processes. Monocytosis also may accompany suppuration, pyogranulomatous and granulomatous inflammation, necrosis, malignancy, hemolytic or hemorrhagic disease, or immune-mediated diseases. Rarely, monocytosis is the only leukogram change in dogs with sepsis or bacterial endocarditis.

Differential diagnosis of monocytosis includes canine response to corticosteroids (see the discussion on stress and corticosteroid response, earlier) and disorders requiring macrophages. Extreme monocytosis may indicate leukemia. If concurrent lymphopenia and eosinopenia are present, a stress or corticosteroid leukocyte response is likely. If lymphopenia and eosinopenia are not present, chronic inflammation or tissue destruction should be suspected.

Monocytopenia is not significant. Low numbers of monocytes normally are present in the blood. Monocytes are unevenly distributed on the blood smear, so they may be undercounted on a 100-cell leukocyte differential count. Uneven monocyte distribution is a greater problem on glass wedge smears than on coverslip smears. Automated cell hematology analyzers often detect more monocytes than microscopic counts.

LYMPHOCYTOSIS

Lymphocytes uniquely retain the ability to divide and recirculate between blood and tissues. Lymphocytosis may be transient (15 to 30 minutes in duration) or persistent. Transient lymphocytosis occurs in physiologic leukocytosis (discussed earlier). Persistent nonneoplastic lymphocytosis usually signifies strong immune stimulation from chronic infection, viremia, immune-mediated disease, or recent immunization. Supportive laboratory evidence of chronic infection (in addition to history and physical findings) may include hyperproteinemia with polyclonal gammopathy; presence of "reactive" lymphocytes; CBC evidence of inflammation; or cytologic or histologic documentation of inflammation, lymph node hyperplasia, or both. Differentiation of persistent extreme lymphocytosis from chronic lymphocytic leukemia (CLL) is discussed later in the chapter.

Reactive Lymphocytes and Blast-Transformed Lymphocytes

Reactive lymphocytes are large, immune-stimulated lymphocytes with dark-blue cytoplasm and irregular, scalloped, or cleaved nuclei. They are also called *immunocytes*. In contrast, blast-transformed lymphocytes have a large nucleus with light, dispersed chromatin pattern with prominent nucleoli or nucleolar rings. Rare reactive lymphocytes are visible in blood smears from healthy animals, whereas a few to several reactive lymphocytes may occur in blood smears from sick or recently vaccinated animals. Reactive lymphocytes are not of special diagnostic significance. The number

of reactive lymphocytes does not consistently reflect the degree of immune stimulation, nor is it pathognomonic for any specific disease. Reactive blast-transformed lymphocytes may appear unusually numerous or active (especially in young animals) and have been mistaken for both lymphoid leukemia and presumed lymphoma with a leukemic blood picture.

LYMPHOPENIA AND EOSINOPENIA

Severe stress or exogenous corticosteroid administration (see Stress and Corticosteroid Response) usually causes lymphopenia and eosinopenia. Lymphopenia is expected early in acute, severe, stressful disease (see Figure 4-5), and the return of the lymphocyte count to normal is a good prognostic sign indicating recovery from the stress of disease. Loss of lymphocyte-rich lymph may cause lymphopenia. Examples include chylothorax in dogs and cats, protein-losing enteropathy and lymphangiectasia in dogs, and disruption of normal lymphatic circulation by inflammatory, infectious, or neoplastic diseases in both species (Latimer, 1995). Lymphopenia occurs in some viral diseases by direct viral damage of lymphoid tissue and through lymphocyte redistribution from stress or antigen exposure. Canine viral diseases causing lymphopenia include distemper, infectious canine hepatitis, parvoviral enteritis, and coronavirus enteritis. Cats may experience lymphopenia in panleukopenia and FeLV infection.

Age affects the lymphocyte count. Younger animals usually have higher counts. For example, the minimal lymphocyte counts expected in dogs of various age categories are 2000/µl from 3 to 6 months of age; 1500/µl from 8 to 24 months of age; and 1000/µl over 24 months of age. Thus one uses different lymphocyte counts to indicate lymphopenia in young animals.

Eosinopenia may be difficult to document by routine WBC counts. These cells may not be observed in feline leukocyte differential counts in health and may account for only 2% of healthy canine leukocyte differential counts. Variation expected with a 100-cell, manual differential leukocyte count when 2% of a cell type is present is 0% to 8% (95% confidence interval). The clinical impression of true eosinopenia in a sick animal is solidified if concurrent lymphopenia is present. The most common causes of eosinopenia are severe stress associated with illness and response to corticosteroid administration. Fictitious eosinopenia of greyhounds and other dogs may result from misidentification of "vacuolated or gray" eosinophils. Instead of having red-orange granules, affected eosinophils appear to have clear vacuoles.

EOSINOPHILIA

General Comments

Eosinophilia usually indicates eosinophilic inflammation somewhere in the body, though intense tissue infiltration with eosinophils may be unaccompanied by detectable eosinophilia because eosinophils have a much shorter half-life in blood than in tissues. Eosinophils kill parasites, regulate the intensity of hypersensitivity reactions mediated by immunoglobulin E (IgE) antibodies, and may promote inflammation and tissue damage (Center et al, 1990; McEwen, 1992). Eosinophils kill parasites by attaching to them and forming a digestive vacuole between the eosinophil and parasite. Eosinophils degranulate potent molecules such as major basic protein and eosinophil peroxidase, which damage the wall of the parasite or ova. More intense eosinophilic response occurs with parasites within tissues (e.g., heartworms, strongyles, migrating lung flukes such as *Paragonimus kellicotti)*. Endoparasites, such as *Giardia* species or tapeworms that do not invade tissue generally do not incite eosinophilia. Some parasites may not stimulate eosinophilia until they die and expose previously hidden antigens. Production of eosinophilia is an immune response. The first exposure to a parasite produces a modest, delayed eosinophilia. The second exposure to the same parasite results in an intense, dramatic eosinophilia.

The inflammatory response to certain allergens is similar to that for tissue-invasive parasites. Lymphocytes respond to the allergen by producing an IgE-type immune response. Eosinophils stimulated to degranulate may destroy normal tissue. For example, eosinophilic inflammation from inhaled allergens in feline asthma may cause damage to respiratory epithelium.

Eosinophils are attracted to inflamed tissue by mast cell products and lymphokines. Eosinophils may be visible in mast cell tumors and lymphoma. The interaction among lymphocytes, mast cells, and eosinophils is extensive. Although mediators of each cell type act locally or on the bone marrow,

T lymphocytes play a major role in eosinophil production and maturation via interleukin-5 production.

Diagnostic Approach

One should consider parasitic and allergic processes first when identifying the cause of eosinophilia (Figures 4-6 and 4-7) (Center et al, 1990; Latimer, 1995). Less frequent causes include neoplasia (e.g., mast cell tumor, lymphoma, mucinous carcinomas, fibrosarcomas, eosinophilic leukemia), fungal infection (e.g., zygomycosis, cryptococcosis), viral infection (e.g., FeLV), or bacterial infection (e.g., *Streptococcus* or *Staphylococcus* species). The hypereosinophilic syndrome can produce severe, persistent circulating and tissue eosinophilia.

Mast cells are numerous near the surface of the skin, digestive tract, respiratory tract, and genitourinary tract; therefore eosinophilia can also be evaluated via a body system approach. The integumentary system is easily examined for ectoparasites, dermatitis, or masses. Flea bite allergy is a common cause of eosinophilia in cats. The respiratory tract may be evaluated cytologically by transtracheal wash, bronchial brushings, swabs, or collecting material adherent to endotracheal tubes. Heartworm disease and pulmonary

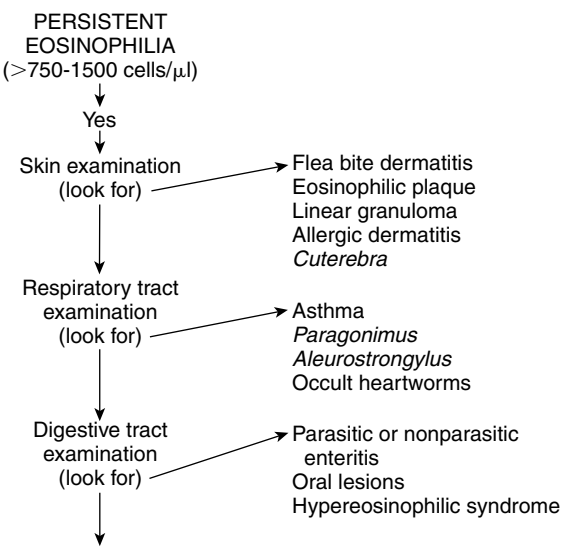

FIGURE 4-6. Evaluation of feline eosinophilia. An algorithm to diagnose common causes of eosinophilia in cats.

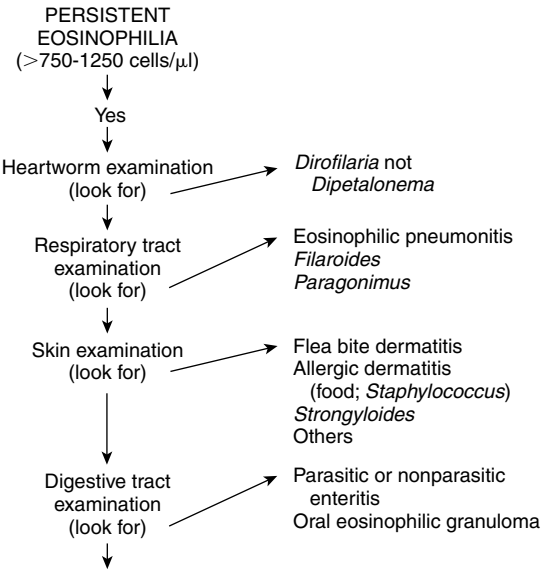

FIGURE 4-7. Evaluation of canine eosinophilia. An algorithm to diagnose common causes of persistent eosinophilia in dogs.

infiltrates with eosinophils (PIE) are common causes of eosinophilia in dogs. Fine-needle aspiration or surgical biopsy may aid in evaluating dermal or pulmonary masses. Examination of the upper alimentary tract should include visual inspection of the oral cavity for eosinophilic granulomas, especially the eosinophilic granuloma complex of cats and Siberian husky dogs. Fecal ova examination is inexpensive, though the presence or absence of ova is usually unrelated to the primary cause of eosinophilia. If eosinophilic gastroenterocolitis is suspected, consider endoscopic examination, surgical biopsy, and cytologic evaluation of any lesions. Estrus in dogs occasionally causes eosinophilia.

BASOPHILIA

Basophils are rare in normal dogs and cats. Basophilia is the presence of greater than or equal to 2% basophils or repeated detection of any basophils. Basophilia of 2% may be missed. Basophilia must be 3% to 6% to be consistently detected. Basophilia may also be missed because basophils are difficult to identify. Feline basophil granules are numerous, moderately sized, round to oval, and grayish beige to light lavender. This appearance

of feline basophils is unique, and these cells may be mistaken for eosinophils with faded granules or monocytes. Feline eosinophils have red-orange, rod-shaped granules, whereas monocytes have blue-gray cytoplasm that is devoid of distinct granules. Infrequently, feline basophils have one or two dark-staining purple granules that facilitate cell identification. Canine basophils have few, widely scattered purple (i.e., metachromatic) or poorly stained granules. These cells may be misidentified as monocytes. The scarcity of basophils in blood smears may contribute to the lack of confidence in identifying these cells. Automated hematology analyzers (i.e., flow cytometers: Advia 120, Cell Dyn 3500) have not been validated to determine accurately feline and canine basophils on automated differential counts.

Basophils are an integral component of hypersensitivity reactions, as are mast cells. Basophils are involved in hemostasis, lipid metabolism, parasite rejection (e.g., ticks), and tumor cell killing (Latimer, 1995). Basophilia has two major causes: (1) parasitism (especially *Dirofilaria immitis* infection) and (2) hypersensitivity reactions. Occasionally, basophilia is associated with lipemia, canine and feline mast cell tumors, or as an unusual component of granulocytic leukemia (Latimer, 1995; O'Keefe et al, 1987).

ABNORMAL NUCLEAR MORPHOLOGY AND CYTOPLASMIC INCLUSIONS

Hyposegmentation is decreased nuclear segmentation of granulocytes, indicating immaturity. Hyposegmentation most commonly indicates inflammation. Hypersegmentation presents with excessive lobulation, often more than five segments. This nuclear change reflects more mature neutrophils. Hypersegmentation may be the result of prolonged neutrophil time in circulation, which is commonly associated with corticosteroid administration. However, genetic developmental abnormalities, preleukemic or dysplastic conditions, and leukemia may be responsible for nuclear changes. Dysplasia and leukemia will be discussed later in this chapter.

Cytoplasmic inclusions may be associated with toxic change in neutrophils. Infectious agents such as bacteria (i.e., neutrophilic *Ehrlichia*), protozoa (i.e., *Hepatozoon canis*, *Leishmania* spp.), or yeast (i.e., *Histoplasma capsulatum*) are found within the cytoplasm of circulating leukocytes providing a definitive diagnosis. Rarely, genetic diseases are responsible for a variety of cytoplasmic inclusions.

Pelger-Huët Anomaly

Pelger-Huët anomaly (PHA) is an inherited disorder of leukocyte development characterized by nuclear hyposegmentation of neutrophils, other granulocytes, and monocytes. Almost all neutrophils resemble bands or metamyelocytes (i.e., absolute numbers look like a severe degenerative left shift). The characteristic WBC appearance should be recognized and reported to avoid undue concern to clinicians reading the CBC who may otherwise consider inflammation or dysplastic changes associated with leukemia. The nuclear chromatin pattern of the granulocytes appears coarse and mature, and the cytoplasm is clear and devoid of toxic changes. PHA presents as a lifelong "degenerative left shift" but is usually an incidental finding. Neutrophils function normally, and a predisposition to infection has not been demonstrated. PHA occurs in both dogs and cats and is presumed to be inherited in an autosomal dominant manner (Latimer, 1995; Latimer and Robertson, 1994). However, PHA may be inherited in an autosomal incompletely dominant pattern in Australian shepherds, suggesting that the expression of the anomaly is governed by two or more alleles (Latimer, Campagnoli, and Danilenko, 2000).

Bacterial infection, drug treatment, developing granulocytic leukemia, or FeLV infection may cause acquired nuclear hyposegmentation of neutrophils (i.e., pseudoPHA). Bona fide PHA anomaly can be confirmed by finding the anomaly in blood smears of parents, siblings, or other relatives or by proving inheritance of the trait by prospective breeding trials. Persistent nuclear changes on repetitive blood smear analysis after eliminating causes of pseudoPHA allows tentative diagnosis.

Genetic Disease with Cytoplasmic Inclusions

Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome (CHS) occurs as an autosomal recessive trait in Persian cats with yellow-green eyes and a diluted smoke-blue haircoat (Latimer and Robertson, 1994). This anomaly is transmitted in an autosomal recessive pattern. This disorder is associated

with large pink lysosomal granules or cytoplasmic inclusions in neutrophils. Coarse clumping of melanin granules is associated with color dilution of the haircoat and irises. In addition, decreased choroidal pigment causes a red fundic reflex and photophobia in bright light. Blood smear examination and the cat's gross appearance confirm diagnosis of CHS. Neutrophil inclusions are positive on peroxidase and Sudan black B stains. In contrast with other animal species, cats with CHS have no predisposition to infection. Cats with CHS have an increased bleeding time secondary to mild platelet dysfunction, however. Hemostasis may be prolonged slightly after trauma, venipuncture, or elective surgery.

Lysosomal Storage Diseases

Storage diseases are rare inherited enzyme defects that cause accumulation of intermediate metabolites of complex molecules within cellular lysosomes. Depending on the nature of the metabolite and its affinity for Romanowsky-type stains, cellular inclusions may appear purple and stringy, as in mucopolysaccharidosis (MPS) VI (i.e., Maroteaux-Lamy syndrome) of cats or MPS VII of dogs, or may appear as clear vacuoles in leukocytes in cats with lysosomal acid lipase deficiency. The characteristic purple granules of MPS can be differentiated from toxic granulation, because cytoplasmic basophilia is absent and toxic granulation is uncommon in dogs and cats. Lysosomal storage diseases often are associated with progressive central nervous system or skeletal disease but may be identified on a CBC by observing characteristic inclusions or vacuoles in circulating leukocytes.

LEUKOCYTE FUNCTION DEFECTS

Neutrophil dysfunction has been reviewed (Latimer, Campagnoli, and Danilenko, 2000). Neutrophil dysfunction is rare but may be suspected in animals with a history of recurrent infections in association with a normal to extremely elevated neutrophil count and lack of neutrophil migration into sites of infection as determined by cytology. Diagnosis of neutrophil dysfunction requires excluding common causes of immune deficiency and documenting abnormalities in neutrophil adherence, chemotaxis, phagocytosis, bactericidal activity, or a combination thereof. Tests of neutrophil function are labor intensive, expensive, and available only through a few specialists or research laboratories to which the animal must be referred for diagnosis.

Canine CD11/CD18 Adhesion Protein Deficiency

Canine adhesion protein deficiency (i.e., canine leukocyte adhesion molecule deficiency, CLAD, canine granulocytopathy syndrome) occurs infrequently in Irish setter and Irish setter-cross puppies. CLAD is an autosomal recessive disease. Affected puppies have recurrent bacterial infections before 12 weeks of age. Leukogram abnormalities often include a leukemoid reaction (e.g., up to 208,000 cells/μl). Fluids from sites of infection contain few neutrophils, because they are less able to immigrate from the microvasculature to the sites of infection. Neutrophils lack CD11/CD18 adhesion proteins on the plasma membrane surface, which facilitate phagocytosis of organisms and cell emigration from blood vessels (Trowald-Wigh et al, 1992).

Chronic Rhinitis and Pneumonia in Doberman Pinschers

Chronic respiratory disease in eight closely related Doberman pinschers has been attributed to impaired neutrophil bactericidal activity (Breitschwerdt et al, 1987). Neutrophils phagocytize bacteria normally but are unable to kill them. Immunoglobulin concentrations, complement concentrations, and mitogen-stimulated lymphocyte transformation are normal.

Acquired Neutrophil Dysfunction

Acquired neutrophil dysfunction has been reported in a few dogs with poorly regulated diabetes mellitus, pyoderma, demodicosis, protothecosis, and lead toxicosis. In cats, neutrophil dysfunction has been documented infrequently in FeLV infection and feline infectious peritonitis (Latimer et al, 2000).

NEOPLASTIC AND DYSPLASTIC DISEASES OF BLOOD LEUKOCYTES

General Comments

The proliferative responses of leukocytes previously discussed arise from the purposeful need to replace missing circulating cells primarily related to increased utilization or consumption. One evaluates the bone marrow

and interprets these changes as hyperplasia. Other proliferations may be characterized as poorly regulated with abnormal cell development, termed *dysplasia*, or as nonpurposeful, unregulated new growth, termed *neoplasia*. Dysplastic changes may be observed in hematopoietic cells with or without neoplasia. These changes may occur as *preleukemia* (i.e., before the onset of overt leukemia) or be observed as an inheritable condition without consequence.

Inherited dysplastic conditions exist in breeds such as toy and miniature poodles with enlarged erythrocytes and their precursors or cavalier King Charles spaniels with abnormally large platelets related to an autosomal recessive defect. These dogs have no clinical signs and the dysplastic findings are usually incidental. Secondary myelodysplasia is more often associated with drug-induced and nutritional conditions that may be reversible once the inciting cause is removed or associated with certain infections, malignancies, and immune-mediated disease (Table 4-6). Idiopathic or primary myelodysplastic syndrome (MDS) is a clonal disease, indicating it originates from a single transformed stem cell that affects multiple nonlymphoid lineages such as erythroid, granulocytic, and megakaryocytic precursors. Any of the primary or secondary forms of myelodysplasia may progress to leukemia.

Leukemia, literally meaning white blood, is a term that reflects an abnormal population of hematopoietic cells found within the blood or bone marrow as opposed to solid tissues, such as lymph node, spleen, or liver. Lymphoid or myeloid (nonlymphoid) cells may be involved in leukemia. Five causes of leukemia are (1) virus infections, such as FeLV that may affect progenitor cell development; (2) genetic abnormalities inherited or acquired that lead to altered cell growth; (3) defective immune systems, such as with FIV infection that does not permit normal defense mechanisms against leukemia; (4) chemicals (e.g., benzene, therapeutic drugs), which have rarely been associated with leukemia; and (5) radiation that may alter progenitor cells.

Clinical signs of leukemia vary. Common signs include lethargy, pale mucous membranes, anorexia, weight loss, fever, frequent infections, icterus, and abnormal bleeding such as petechiae. Physical findings often include hepatosplenomegaly, lymphadenopathy, or tonsillar enlargement.

Strong laboratory indicators of leukemia include marked leukocytosis of a monomorphic cell type, the presence of many blast cells, or morphologic irregularities in shape or size of late-stage forms. A large buffy coat may be noted when measuring the PCV because of marked leukocytosis or thrombocytosis. Blast cells have a large round nucleus, fine chromatin pattern, and one or more prominent nucleoli. Reactive and blast-transformed lymphocytes are often present in small numbers (e.g., 1 to 5/blood smear) and are not diagnostic. Numerous reactive and blast-transformed lymphocytes may occur in ill, nonleukemic animals (e.g., 1% to 5% of WBC) and may mimic leukemia. Rare mitotic figures do not indicate leukemia because mitotically active cells such as rubricytes, promyelocytes, myelocytes, monocytes, and reactive lymphocytes are encountered in nonleukemic animals. Morphologic irregularities may include developmental changes of *dysplasia*, such as megaloblastic erythroid precursors that have an excessive amount of cytoplasm and unusual nuclear chromatin pattern, enlarged segs with many nuclear lobes (i.e., macropolycytes), enlarged highly vacuolated platelets, or dwarf megakaryocytes, and *asynchronous maturation* wherein maturity of nucleus and cytoplasm differ.

TABLE 4-6. Forms of Myelodysplasia and Associated Conditions

GENERAL CATEGORY	ASSOCIATED CONDITIONS
Congenital dysplasia	Toy and miniature poodles (erythrocytes and precursors) Cavalier King Charles spaniels (platelets) Giant schnauzers (neutrophilic precursors)
Secondary MDS	Drugs: azathioprine, cyclophosphamide, cytosine arabinoside, vincristine, chloramphenicol Infectious agents: FeLV, FIV Nutritional deficiencies: folate, cobalamin receptor defect in giant schnauzers Immune-mediated disease: immune-mediated anemia/thrombocytopenia Malignant diseases: lymphoma, chronic myeloproliferative disease, plasma cell myeloma
Primary MDS	Idiopathic clonal defects

MDS, Myelodysplastic syndrome; *FeLV*, feline leukemia virus; *FIV*, feline immunodeficiency virus.

White cell counts in leukemia are variable from leukocytosis, leukopenia, or even within normal limits. Anemia is often nonregenerative but rarely hemolysis is found in some forms of leukemia. Thrombocytopenia or less commonly thrombocytosis is associated with the other hematologic changes. Laboratory tests used to diagnose hematopoietic neoplasia or myelodysplasia and evaluate the extent of involvement include bone marrow evaluation, biochemical profiles, cytology, serology, and urinalysis, in addition to hematology. If changes in the blood are not diagnostic, bone marrow examination may confirm leukemia. Blast cells are often found in much higher numbers in marrow than in blood. Bone marrow evaluation should involve optimally an aspirate and core biopsy with concurrent peripheral blood evaluation. Cytology of effusions or solid tissue masses help to determine a primary source. Serology is indicated for evaluation of leukemia-causing viruses. Protein evaluation of the serum and urine is helpful in determining the presence of gammopathies associated with lymphoproliferative conditions (see Chapter 12).

NOTE: Leukemia is less common than other causes of alterations in leukocyte numbers and morphology. Stress, inflammation, and reactive lymphocytes are much more common.

When the diagnosis of leukemia is confident, without knowledge of the specific type, many veterinarians stop the workup and recommend euthanasia. The following information is for those choosing to treat leukemic cases and who need a more specific diagnosis for treatment choices. Definitive diagnosis of specific leukemias usually requires a veterinary clinical pathologist. Slide review from routine CBC with Wright's-stained smears of EDTA anticoagulated blood may allow differentiation of the cellular origin, such as lymphoid from myeloid (i.e., nonlymphoid). Evaluation of the bone marrow is often necessary to classify the leukemia, because the blood may not be representative of the degree of leukemic involvement. If cell morphology in a leukemic patient is insufficient to determine the cell lineage, one may use specialized tests (i.e., cytochemistry, electron microscopy, immunocytochemistry) usually conducted at academic institutions or referral laboratories. Cytochemistry involves nonimmunologic cytoplasmic markers for enzymes, lipids, or glycogen within the cell (Raskin and Valenciano, 2000). Enzymes include peroxidase found in certain granulocytes and nonspecific esterases found in monocytes or T lymphocytes (see Table 4-7).

TABLE 4-7. Selected Cytochemical Stains for Identification of Cell Types in Canine and Feline Leukemias

STAIN	CELLS THAT STAIN
Peroxidase	Neutrophils—all stages positive Monocytes—weak positive when present Eosinophils—positive in dog, negative in cat
Sudan black B	Neutrophils—all stages positive Monocytes—weak positive when present Eosinophils—positive in dog, negative in cat
Leukocyte alkaline phosphatase	Neutrophils—myeloblasts, strong Monocytes—monoblasts, weak or rare in dogs Lymphocytes—subset of B cells Eosinophils—between the granules in cat Basophils—weak or occasional staining
Chloroacetate esterase	Neutrophils—strong positive Basophils—moderately positive Mast cells—variably positive Megakaryocytes—weak positive when present
Alpha-naphthyl butyrate esterase	Monocytes—diffuse positive Lymphocytes—focal positive in T-cells Megakaryocytes—diffuse positive
Nonspecific esterase + fluoride*	Lymphocytes—positive in dog, variable in cat Megakaryocytes—weak positive in dog
Periodic acid-Schiff	Neutrophils—all stages positive Megakaryocytes—positive in dog, variable in cat Mast cells—variably positive Lymphocytes—plasma cells Eosinophils—weak or variable staining Basophils—weak or variable staining Monocytes—weak or variable staining
Acid phosphatase	Neutrophils—all stages positive Lymphocytes—focal positive in T-cells Eosinophils—positive Basophils—positive in cat, variable in dog Monocytes—diffuse positive Megakaryocytes—diffuse positive Mast cells—positive

*Inhibition of nonspecific esterase with fluoride is used to inhibit or reduce the staining of monocytes.

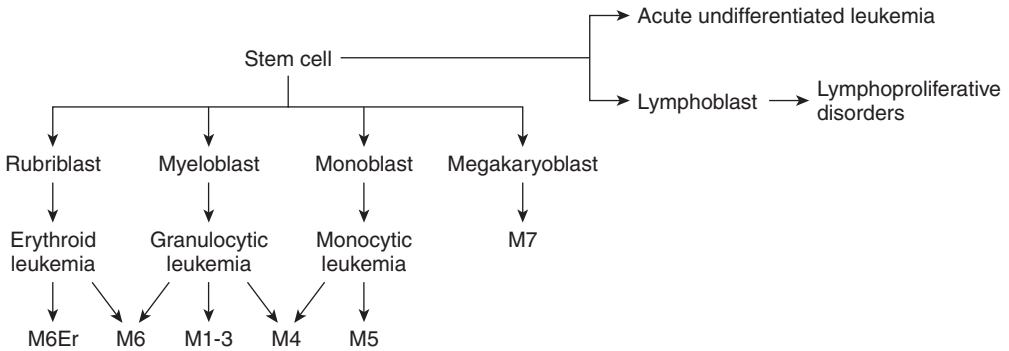

FIGURE 4-8. Diagram depicting origin of the most common neoplastic disorders of hematopoietic cells. *M6Er*, Erythroleukemia with erythroid predominance; *M6*, erythroleukemia; *M1-3*, myeloblastic and promyelocytic leukemias; *M4*, myelomonocytic leukemia; *M5*, monoblastic leukemia; *M7*, megakaryoblastic leukemia.

Electron microscopy is helpful to study the ultrastructural features of the cytoplasm and nucleus (Grindem, Perman, and Stevens, 1985a and 1985b). Immunocytochemistry involves staining of specific cell surface antigens by the use of antibodies such as monoclonal antibodies against CD3 (T cells), CD4 (helper cells), CD8 (cytotoxic or suppressor cells), CD14 (monocyte progenitors), CD21 (B cells), and CD79 (B cells) (Moore and Vernau, 2000). Blood and bone marrow specimens for these special procedures require specific instructions in handling, preparation, and fixation. Therefore the clinical pathologist should be consulted to avoid delay in diagnosis caused by improper sample or sample handling.

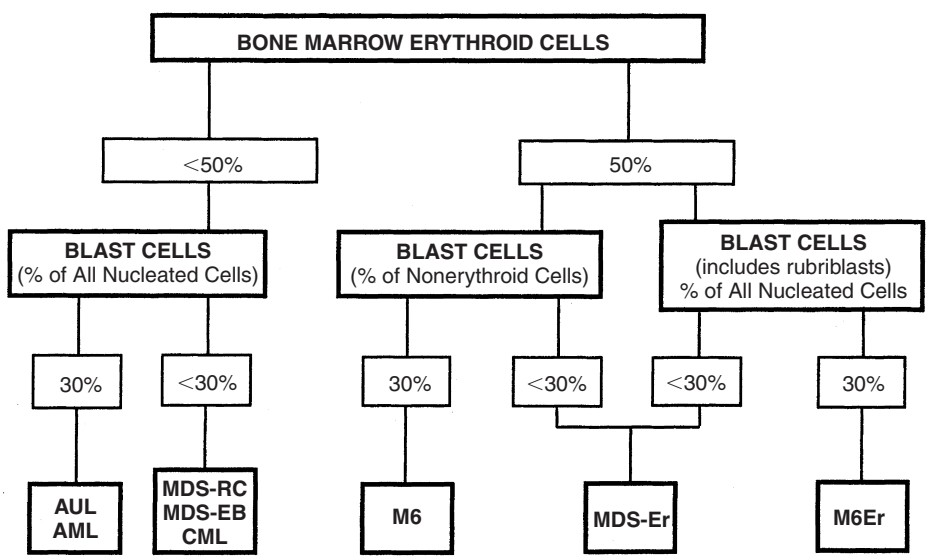

FIGURE 4-9. A scheme to classify myeloid leukemias in dogs and cats using the bone marrow erythroid cells from all nucleated cells *(ANC)*. ANC is a group that excludes lymphocytes, plasma cells, macrophages, and mast cells. Nonerythroid cells are calculated as ANC minus erythroid cells. Blast cells include myeloblasts, monoblasts, and megakaryoblasts. *AUL*, Acute undifferentiated leukemia; *AML*, acute myeloid leukemias M1-M5 and M7; *CML*, chronic myeloid leukemias; *MDS*, primary myelodysplastic syndrome; *RC*, refractory cytopenia; *EB*, excess blasts; *MDS-Er*, myelodysplastic syndrome with erythroid predominance; *M6*, erythroleukemia; *M6Er*, erythroleukemia with erythroid predominance. (Reprinted with permission from Raskin RE, *Vet Clin North Am Small Anim Pract* 26:1023, 1996.)

BOX 4-1. Summary List of Important Lymphoproliferative and Myeloproliferative Disorders

LYMPHOPROLIFERATIVE DISORDERS

Preneoplastic:

Persistent Lymphocytosis

Leukemic/Disseminated Forms:

Lymphoblastic Leukemia (B-, T-, Null-Cell Types)
Lymphocytic Leukemia (B-, T-Cell Types)
Granular Lymphocyte Leukemia (T-, NK-Cell Types)
Plasma Cell Myeloma
Mycosis Fungoides/Sézary Syndrome

Neoplastic Solid Tissue Forms:

Lymphoma (B- and T-Cell Types of Nodal and Extranodal Sites)
Plasmacytoma (Extramedullary Sites)

MYELOPROLIFERATIVE DISORDERS*

Preneoplastic:

Myelodysplastic Syndrome (Primary)

Leukemic/Disseminated Forms:

Acute Myeloid Leukemia (Types M1-M7)

Chronic Myeloproliferative Disease†

Chronic myelogenous leukemia (neutrophils)
Eosinophilic leukemia
Basophilic leukemia
Chronic myelomonocytic leukemia
Polycythemia vera
Essential thrombocythemia
Myeloid metaplasia/myelofibrosis or idiopathic myelofibrosis

Mast Cell Leukemia/Mastocythemia†

Malignant Histiocytosis or Disseminated Histiocytic Sarcoma (Dendritic Cells)

Neoplastic Solid Tissue Forms:

Cutaneous Histiocytoma (Dendritic Cells)
Localized Histiocytic Sarcoma (Dendritic Cells)

*The term *myeloproliferative* is used in its broadest sense to include nonlymphoid dysplastic and neoplastic conditions.
†Uncommon disease states.

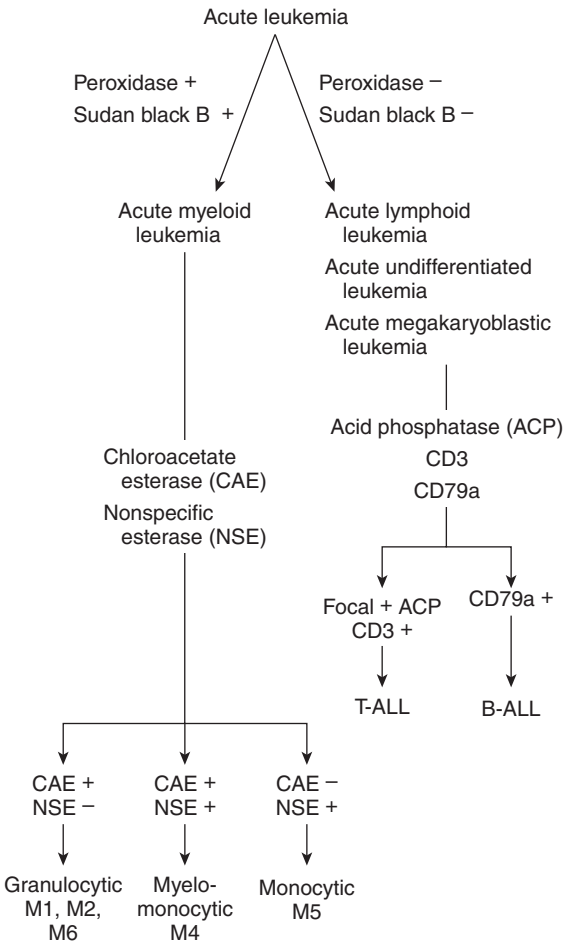

FIGURE 4-10. Simple classification of acute leukemia using cytochemical stains and immunocytochemistry.

Classification Schemes

Classification schemes for hematopoietic neoplasms are based primarily on cell lineage as determined by examination of Romanowsky-type (e.g., Wright's-) stained blood and bone marrow smears (Figures 4-8 and 4-9). Initially, hematopoietic neoplasms are separated into lymphoproliferative and myeloproliferative disorders (Box 4-1). The lymphoproliferative disorders involve lymphocytes, whereas myeloproliferative disorders involve the remaining nonlymphoid leukocytes, erythroid cells, and megakaryocytes or platelets singly or in combination. The distinction may be determined by cytochemical staining (Figure 4-10).

Various classification systems also have been used to classify lymphoid neoplasms. Most of these systems, such as the National Cancer Institute Working Formulation and updated Kiel System, rely on morphologic and immunologic features. Recently a system based on the World Health Organization classification of human lymphoid tumors has been suggested for animals (Valli et al, 2002). This veterinary system separates lymphoid neoplasms by immunophenotype and tumor site location primarily supported by morphologic criteria and may provide better prognostic information than previous classification schemes. Histopathology plays a key role in using this system. The lymphoid leukemic forms are

classified as acute or chronic. An abundance of blast cells or immature cells characterizes *acute* leukemia. *Chronic* leukemia has a proliferation of differentiated cells that may be difficult to distinguish from hyperplasia (e.g., reactive response). Generally, chronic leukemias have a more favorable prognosis than acute leukemias and therefore survive for longer periods of time.

Morphologic criteria to classify acute myeloid leukemias (AMLs) in dogs and cats have been developed by the American Society for Veterinary Clinical Pathology (ASVCP) (Jain et al, 1991). The percentage of blast cells was suggested as equal to or greater than 30% of nonlymphoid and nonhistiocytic cells in the bone marrow for AML, whereas chronic myeloid leukemias, MDSs, and leukemoid reactions would be expected to have less than 30% blast cells (see Figure 4-9). The AML are further separated and subtype is assigned (M1-M7) depending on the cell type (see Figure 4-10). Adjectives (e.g., granulocytic or myelogenous, monocytic, lymphocytic; see Figure 4-8) are used to designate the cell lineage. If the cell type cannot be determined, it is simply called an *acute undifferentiated leukemia*.

NOTE: In general, lymphoproliferative disorders are more responsive to treatment than acute myeloproliferative disorders.

Lymphoproliferative Disorders

Lymphoproliferative disorders are lymphoid conditions originating from solid tissues, such as the lymph nodes (i.e., nodal), alimentary tract, thymus, spleen, eye, and skin or from the bone marrow, such as plasma cell myeloma or some forms of leukemia. A persistent increase in differentiated lymphocytes over time without reaction to known infectious agents or antigenic stimulants suggests neoplasia in lieu of immune stimulation. Lymphoid neoplasms arranged in descending frequency of occurrence are lymphoma, plasma cell myeloma, CLL, and acute lymphoblastic leukemia (ALL). The approximate annual incidence rate for leukemias in dogs and cats is 31 and 224 cases per 100,000 animals, respectively. Lymphoid malignancies, including lymphoma, are responsible for over 85% of cat leukemias and over 65% of the dog leukemias. Cats have a sixfold greater incidence of lymphoma and a sixteenfold greater incidence of myeloproliferative disease than dogs, primarily the result of induction of neoplasia by FeLV infection.

Nodal Lymphoma

Lymphoma, formerly lymphosarcoma, is the most common lymphoid neoplasm of dogs and cats. Lymphoma usually originates in peripheral lymphoid tissues but may develop within extranodal sites (e.g., skin) or from precursor cells in the bone marrow. Multicentric disease is more common in dogs and usually involves the peripheral lymph nodes, spleen, and liver. In descending frequency, other forms of canine lymphoma include alimentary, thymic, and cutaneous disease. Thymic lymphoma is most common in young cats, whereas alimentary lymphoma is more common in older cats. Cats may also develop multicentric, renal, and cutaneous lymphoma. About 60% to 80% of cats with the thymic or multicentric forms of lymphoma are positive for FeLV group-specific antigen, whereas only 30% of cats with alimentary lymphoma are positive.

The CBC is not sensitive in identifying canine lymphoma; 21% of affected dogs have lymphocytosis and 25% have lymphopenia. At the time of diagnosis, up to 57% of dogs may be leukemic. Approximately 30% of cats with lymphoma have a leukemic blood profile. The CBC may document concurrent cytopenias, including anemia, thrombocytopenia, and leukopenia. Bone marrow involvement is used in staging lymphoma and determining treatment. Bone marrow evaluation is needed, because CBC results may not reflect marrow involvement and vice versa. Bone marrow core biopsies are most sensitive in identifying leukemic involvement (e.g., 97% of canine cases compared with 50% by blood smear analysis alone or compared with 60% using bone marrow aspirate biopsies alone) (Raskin and Krehbiel, 1989). Hypercalcemia (i.e., > 12 mg/dl), a factor most associated with T-cell lymphomas (see Chapter 8) is helpful in directing diagnostic efforts toward a lymphoid malignancy. Monoclonal gammopathy may occur infrequently with B-cell lymphoma.

Lymphoma is diagnosed daily by cytopathologists (see Chapter 16). Fine-needle aspirates of lymph nodes or other affected tissues and organs usually disclose a homogeneous population of lymphoblasts. If cytology is equivocal in distinguishing

extreme lymphoid hyperplasia from early lymphoma, a surgical biopsy may provide a definitive diagnosis based on alterations of normal tissue architecture. Surgical or endoscopic biopsies of deep tissues also may be diagnostic.

Immunophenotyping is useful for prognostic evaluation of lymphoma cases; dogs with T-cell lymphoma are at significantly higher risk of relapse and early death compared with B-cell lymphoma. Diagnostic immunocytochemistry of fine-needle aspirate material (or by flow cytometry) and immunohistochemistry of tissue sections are performed currently at certain academic institutions (e.g., flow cytometry at University of California at Davis and North Carolina State University).

In contrast to the mediastinal or thymic lymphoma that is composed of neoplastic lymphocytes and most associated with young cats and dogs, a neoplasm of thymic epithelium, termed *thymoma*, contains a variable numbers of mature lymphocytes and is usually observed in older dogs and cats. Both neoplasms will present as a cranial mediastinal mass, often with evidence of coughing, dyspnea, or thoracic effusion, including chylothorax. Thymic tumors are associated with paraneoplastic hypercalcemia. Thymoma may involve a concurrent mature lymphocytosis exceeding 25,000/µl blood and may be confused with CLL. Thymomas are potentially treatable. Surgical biopsy is the preferred method of diagnosis for thymoma as cytology may be confusing because of secondary chylothorax or the presence of many mast cells.

Plasma Cell Myeloma

Plasma cell myeloma (PCM) is a lymphoproliferative disorder of bone marrow plasma cells. Extramedullary plasmacytoma occurs in soft tissues (e.g., liver, kidney, spleen, skin). Four diagnostic features of PCM are (1) hyperproteinemia with a monoclonal gammopathy, (2) osteolytic lesions in spine, (3) greater than 15% to 20% plasma cells in bone marrow aspirates, and (4) Bence Jones proteinuria (see Chapters 7 and 12). Bence Jones proteinuria is infrequent in dogs and cats, and these light chains of antibodies are best identified by electrophoresis of urine with *detectable* proteinuria. Ehrlichiosis may mimic plasma cell neoplasia in having strong plasmacytosis in the bone marrow and an oligoclonal gammopathy in serum resembling a monoclonal gammopathy.

Histologic or cytologic evaluation of osteolytic areas or soft tissue masses is most diagnostic of PCM (see Chapter 16). Neoplastic plasma cells may exhibit anisocytosis, anisokaryosis, and a finely dispersed chromatin pattern. Binucleate plasma cells occur frequently in PCM, but binucleated plasma cells are normally visible in hyperplastic lymphoid tissue (e.g., lymph node aspirate). Plasma cell leukemia (i.e., neoplastic plasma cells in blood) occurs infrequently.

Acute Lymphoblastic Leukemia

ALL is less frequent than lymphoma. This rapidly progressive condition occurs mostly in middle-aged dogs (mean 6 years) and frequently in FeLV-infected cats. Because leukemia originates in the bone marrow, ALL may be associated with variable nonregenerative anemia, neutropenia, and thrombocytopenia in any combination. As the condition advances, lymphoblasts are found in enlarged visceral organs such as the liver and spleen. In ALL, lymphocyte size on the stained blood smear varies, with many lymphoblasts. CBC usually allows diagnosis of ALL; however, bone marrow is more consistently abnormal (i.e., dense infiltration by lymphoblasts). Lymphoblasts are difficult to distinguish from poorly differentiated myeloid precursors without cytochemical stains, cell surface markers, or PCR clonality tests, currently used as a research tool (see Figure 4-10). Most feline ALL cases have the T-cell immunophenotype, but canine cases may be T-, B-, or null-cell phenotypes. Hypercalcemia is an infrequent laboratory finding.

Reactive or blast-transformed lymphoid cells on blood smears, especially in young animals undergoing strong immune stimulus (e.g., infections), may confuse the diagnosis of ALL or other leukemias. In many nonneoplastic conditions (e.g., immune stimulation by illness or vaccination), a few to several reactive lymphocytes and blast-transformed lymphocytes (perhaps five per blood smear) may be observed. In contrast, ALL has a predominance of lymphoblasts and perhaps five lymphoblasts per oil immersion field. The use of buffy coat preparations to concentrate the leukocytes to find a few lymphoblasts is not advised, because these preparations often have several blast-transformed lymphoid cells in normal animals.

Chronic Lymphocytic Leukemia

CLL has a relatively favorable prognosis of continued life for about 12 months after diagnosis. It occurs mostly in middle- to old-aged dogs (mean 10 years) with a higher frequency in females. Cats rarely have this form of leukemia; most are FeLV negative. Differentiation of CLL from ALL or lymphoma with a leukemic blood profile is based on the relatively mature appearance of lymphocytes in CLL versus finding lymphoblasts in the other conditions. Lymphocytes in CLL are larger than normal and more homogeneous in appearance than in normal animals. Diagnosis of CLL is aided greatly by magnitude of the lymphocytosis, which may exceed 40,000 to 100,000 lymphocytes/µl. Maximum lymphocytosis in extreme immune reactions (e.g., chronic canine rickettsial infections) seldom exceeds 25,000 lymphocytes/µl and usually is less than 15,000 lymphocytes/µl. Lymphocytosis in cats with strong persistent, immune stimulus in nonleukemic diseases may reach higher maximum lymphocyte counts (e.g., 40,000/µl). Reactive lymphocytosis in young, asymptomatic cynomolgus monkeys can reach 70,000 lymphocytes for 1 or 2 weeks. These extreme changes may be called a lymphocytic leukemoid reaction. CLL is the first entity to rule out when lymphocytosis exceeds 25,000 to 40,000 lymphocytes/µl. The likelihood of CLL increases proportionally to lymphocytosis; counts greater than 50,000 to 100,000/µl are particularly diagnostic. Mild normocytic, normochromic nonregenerative anemia is common with variable thrombocytopenia.

Fine-needle aspirates of lymph nodes, spleen, and liver in CLL document lymphoid proliferation, but differentiation of hyperplasia from CLL may be difficult. CLL has a more homogeneous population, whereas hyperplasia has a more diverse population of lymphocytes, plasma cells, and lymphoblasts. Biopsy and histopathology may better document infiltration by neoplastic lymphocytes with loss of tissue architecture. In bone marrow aspirates, greater than 15% to 20% lymphocytes suggests CLL.

Immunophenotyping has demonstrated many of these cases to be T-cell in origin in the dog. Dysproteinemia may occur in dogs with B-cell chronic lymphocytic leukemia related to monoclonal gammopathy (most often IgM, but IgA and IgG gammopathies also occur). Another diagnostic test, currently available as a research tool, involves analysis of gene rearrangements to determine clonality and whether the lymphocyte proliferation is malignancy or hyperplasia.

Granular Lymphocyte Leukemia and Lymphoma

A subtype of lymphoid leukemia and lymphoma involves mostly medium-sized cells having abundant light-blue to clear cytoplasm that contain several small red or purple granules, termed *azurophilic granules*. These cells are called large granular lymphocytes (LGL) and are normally present in the blood of many animal species; specifically in the dog, LGL are present in 0% to 5% of leukocytes or in 0% to 19% of lymphocytes. The LGL may be increased as a result of immune-stimulation such as seen in canine ehrlichiosis. However neoplastic proliferations of LGL are common. In the cat, granular lymphocytes associated with intestinal lymphoma have prominent large coarse purple cytoplasmic granules. In the dog, LGL malignancies may appear as leukemia in the blood or as lymphoma in tissues such as the lymph nodes and spleen. In these tissue forms, blood lymphocyte counts can be normal, however neutropenia may be evident. The granules do not stain well with aqueous-based Wright's stains, such as those found in commercial quick stains. However, the granules appear readily with Wright's-Giemsa stain. Immunophenotyping of these cells indicate most are cytotoxic T cells expressing CD3 and CD8.

In the dog, LGL leukemia may be first recognized as a benign nontransitory proliferation of granular lymphocytes in the peripheral blood, termed *persistent lymphocytosis*. These cells are thought to arise from the red pulp of the spleen. As disease progresses, splenomegaly is commonly noted and cytologic aspirates demonstrate a marked increase in the LGL population. Typically, the bone marrow appears normal or contains minimal LGL involvement. Most dogs with LGL leukemia behave like CLL with a slow indolent course over several years, whereas a small group may have a more aggressive course, similar to ALL (McDonough and Moore, 2000).

Cutaneous Lymphoma

Mycosis fungoides is an uncommon form of epitheliotrophic cutaneous lymphoma (ECL) usually beginning in the skin and progressing to involve lymph nodes, spleen, and

bone marrow. A diagnostic feature of mycosis fungoides is focal accumulation of lymphoid cells within the epidermis forming *Pautrier's microabscesses*. *Sézary syndrome* is a rare variant of ECL associated with a leukemic blood profile characterized by large T cells with markedly convoluted nuclei (Latimer and Rakich, 1996). The neoplastic cells of ECL are cytotoxic T-cells that express CD3 and CD8.

In comparison to the epitheliotrophic mycosis fungoides, nonepitheliotrophic lymphoma is less frequent. This form was previously believed to be of B-cell origin; however, recent findings indicate this is exclusively T-cell lymphoma, although the morphologic and immunologic characteristics differ from mycosis fungoides. Both forms are aggressive and respond sporadically or poorly to treatment (Moriello, 2000).

Myeloproliferative Disorders

Characteristics of selective myeloproliferative disorders are described under the following headings. Myeloproliferative disorders include a preleukemic condition with dysplasia and neoplasia of mature and poorly differentiated nonlymphoid cells in the form of leukemia (see Box 4-1). Generally, AMLs are severe, rapidly progressive diseases, essentially unresponsive to treatment. In chronic myeloid leukemia, relatively mature cells are released from the bone marrow and the clinical course may be long. In myeloproliferative disorders, the leukemic cell type may vary over time in the same animal; therefore the diagnosis may reflect a specific point in time.

Blast cells of various cell types often lack identifiable characteristics, and the initial impulse is to call an undifferentiated blast a lymphoblast. Blasts in myeloproliferative disease are distinguished by sequential differentiation into more mature myeloid cells, if present, or cytochemical staining of a poorly differentiated leukemia (see Table 4-7).

NOTE: Myeloproliferative disorders occur infrequently or rarely, in contrast to lymphoproliferative disorders, which are more commonly observed.

Myelodysplastic Syndromes

Primary MDS is an irreversible acquired clonal disorder of multipotential hematopoietic cells in contrast to secondary MDSs, which are related to concurrent disease, nutritional deficiency, or drug-induced toxicosis (see Table 4-6). MDS is sometimes referred to as preleukemia, because patients having this syndrome often suffer from chronic debilitation that may remain unchanged or evolve into AML. Characteristic preleukemic changes in hematopoietic cells include cytopenias of one or more cell lines in peripheral blood and morphologic evidence of dysplasia. MDS has less than 30% myeloblasts of the nonlymphohistiocytic cells in the bone marrow, whereas AML has greater than 30% (see Figure 4-9). Several morphologic types exist relative to the numbers of myeloblasts present (e.g., MDS-RC [refractory cytopenia] or MDS-EB [excess blasts]) and erythroid cells present (e.g., MDS-Er [erythroid predominance]) (Raskin, 1996). Cases of MDS-EB having 5% or greater marrow myeloblasts, demonstrate shorter survival and poor response to treatment.

Bone marrow evaluation should be performed to document dysplasia if laboratory findings reveal macrocytic nonregenerative anemia, multiple cytopenias, or a persistent anemia despite attempted regeneration. Dysplastic changes vary among individuals. The erythroid line may have excessive numbers of rubriblasts and prorubricytes (apparent maturation arrest); megaloblastic rubricytes; nuclei with abnormal chromatin patterns, lobulation, or multinucleation; sideroblasts; or combinations thereof. Normal feline marrow lacks sideroblasts or stainable hemosiderin. The myeloid line may have excessive myeloblasts and promyelocytes (apparent maturation arrest), abnormal granulation, bizarre hypersegmented neutrophils (macropolycytes), nuclear hyposegmentation (acquired Pelger-Huët change), giant neutrophils, monocytoid neutrophils, or combinations thereof. The megakaryocytic line may have dwarf megakaryocytes, megakaryocytes with hypolobulated or multiple round nuclei, megakaryocytes with hyperlobulated nuclei with blue cytoplasm, megaplatelets, hypogranular platelets, hypergranular platelets, or combinations thereof. Multiple CBC over

NOTE: Preleukemic changes in hematopoietic cells include cytopenias of one or more cell lines in peripheral blood and morphologic evidence of dysplasia in blood or bone marrow.

time are needed to monitor the cytopenia or to document conversion to leukemia.

Acute Myeloid Leukemia

AML refers to a collection of neoplastic disease affecting precursors of nonlymphoid cells. Subtypes M1-M7 are defined by the cell of origin and the degree of differentiation (Raskin, 1996). Cytochemical staining (see Figure 4-10 and Table 4-7) or immunocytochemistry is used to distinguish the subtypes. For example, *acute myeloblastic (myelogenous) leukemia* (AML-M2) usually consists of neutrophils, but coproduction of basophils and eosinophils may occur. Total leukocyte counts are variable, but extreme leukocytosis may occur. Blast cells are often found in circulation. Severe anemia and thrombocytopenia may accompany AML. Myeloblasts, promyelocytes, and atypical cells denote subtypes M1 to M3. Myeloblasts may have a moderately basophilic cytoplasm with few small azurophilic granules. A disorderly maturation sequence also suggests neoplasia but may occur during repopulation of the bone marrow after cellular destruction (e.g., toxin, parvovirus). Repopulation of the bone marrow begins with immature cells (i.e., myeloblasts, progranulocytes, myelocytes), without the expected predominance of mature bands and segmenters; this appearance initially resembles leukemia, but progressive maturation eventually restores a normal marrow cell population. Diagnosis may require reevaluation of the bone marrow after a few days.

Myelomonocytic leukemia (M4) is characterized by combined production of neutrophils and monocytes by the bipotential stem cell resulting in myeloblasts and monoblasts that equal or exceed 30% of nonerythroid cells in the bone marrow. This is the most common nonlymphoid leukemia. Percentage of monocytes and neutrophils may change as the disease progresses. Moderately severe anemia is expected.

Monocytic leukemia (M5) occurs infrequently. Cytochemical staining may prevent misdiagnosis of monocytic leukemia as acute lymphocytic leukemia. Monoblasts are increased to 30% or greater of the nonerythroid cells of the bone marrow. Monoblasts have basophilic cytoplasm that lacks any obvious granulation; nuclei are irregularly round, producing a folded or creased appearance.

Erythroleukemia (M6) involves proliferation of immature and atypical erythroid and granulocytic cells. The erythroid component exceeds 50% with myeloblasts and monoblasts (together) equal to or greater than 30% of nonerythroid cells. A variant form called *M6Er*, consists predominantly of erythroid precursors; in this form rubriblasts exceed 30%. The M6 conditions are more common in cats than in dogs. Severe nonregenerative anemia and dysplastic changes are frequently prominent. Over time, this form of leukemia may change in appearance and progress to involve predominantly granulocytic precursors. It is often associated with FeLV infection in cats.

Megakaryoblastic leukemia (M7) has been reported in both dogs and cats. Leukocyte and platelet counts vary, and circulating megakaryoblasts may be found. These cells have a round nucleus and scant basophilic cytoplasm with a ragged irregular cell surface. Platelet morphology is often bizarre, characterized by giantism and abnormal granulation. Diagnosis of this form of leukemia requires positive cytochemical reactions to periodic acid-Schiff, alpha naphthyl acetate esterase, acetylcholinesterase, and factor VIII-related antigen. Electron microscopy may reveal characteristic alpha granules or early internal membrane demarcation systems. Some previously reported cases have been misdiagnosed as AML-M7 that were more correctly identified as myeloid hyperplasia with myelofibrosis (discussed later in the chapter).

Chronic Myeloproliferative Diseases

Chronic granulocytic (myelogenous) leukemia (CGL, CML) differs from acute granulocytic leukemia in that segmented and band neutrophils predominate. The left shift in CGL may extend back to promyelocytes, and bone marrow myeloid proliferation may involve orderly myeloid maturation with a marked increase in the myeloid : erythroid ratio (Fine and Tvedten, 1999). This ratio may fall between 4:1 to 25:1 or as high as 36:1. The bone marrow contains less than 30% myeloblasts of nonlymphohistiocytic cells (see Figure 4-9). Diagnosis of CGL is often based on finding marked, persistent leukocytosis (40,000 to 200,000/µl) and exclusion of a leukemoid reaction, discussed earlier under inflammatory leukocytosis. Anemia is mild-to-moderate and platelet counts are variable. Lymphadenopathy with lymph node aspirates that resemble marked extramedullary hematopoiesis may occur in CGL. The prognosis and response to treatment is better

than for AML, with death occurring months after diagnosis because of a blast cell crisis.

Eosinophilic leukemia is a variant form of myelogenous leukemia. This type of leukemia is rare but has been reported in the dog and documented in the cat associated with FeLV infection. It is characterized by a marked, persistent eosinophilia (often > 50,000/µl) and a shift toward immaturity. A moderate anemia may be present. It may be difficult to differentiate from hypereosinophilic conditions (e.g., hyeneosinophilic syndrome, allergies, parasitism, eosinophilic inflammatory diseases, mast cell tumors, certain lymphomas). Leukemic eosinophils will leave the bone marrow and infiltrate solid tissues, such as the lymph nodes, liver, and spleen.

Basophilic leukemia is another variant form of myelogenous leukemia that is uncommon and reported to occur mostly in dogs. This form of neoplasia can be distinguished from mast cell leukemia by subtle nuclear indentation, segmentation, or lobulation. In dogs, the cytoplasmic granules in neoplastic basophils may be coarser than in mast cells. The disorder has been associated with thrombocytosis and anemia. Prognosis is good with treatment.

Chronic myelomonocytic leukemia (CMML) is an uncommon disorder in which blast cells of both granulocytic and monocytic lines involve less than 30% of nonlymphohistiocytic bone marrow cells. Cases often display peripheral monocytosis greater than 4000/µl. Diagnosis is suspected if marked monocytosis accompanies neutrophilia, without indications of an inflammatory condition. CMML may progress over time to an AML.

Polycythemia vera (primary erythrocytosis) is a rare disease in dogs and cats involving the neoplastic production of mature, anucleated erythrocytes. Polycythemia is suggested by brick-red mucous membranes related to markedly increased hematocrits (i.e., PCV 65% to 82%). Splenomegaly if present is mild. Polyuria, polydipsia, hemorrhage, and neurologic disorders occur in 50% of canine cases. Definitive diagnosis of polycythemia vera requires ruling out other causes of erythrocytosis or absolute polycythemia (see Chapter 3). Diagnosis is based on demonstration of an absolute increased red cell mass, normal PaO_2, and a decreased serum erythropoietin concentration that is measured at specialized laboratories. Renal cysts, pyelonephritis, and tumors must be excluded, because they can also produce absolute polycythemia (i.e., inappropriate secondary erythrocytosis) as a paraneoplastic syndrome with increased serum erythropoietin concentration.

Essential thrombocythemia (i.e., primary thrombocythemia) is a rare chronic myeloproliferative disease characterized by proliferation of megakaryocytes and unregulated platelet production reported in the dog and cat. It occurs unrelated to physiologic or responsive thrombocytosis. Clinical signs include splenomegaly and platelet function abnormalities, such as spontaneous bleeding and thromboembolism (see Chapter 5). Platelet counts are persistently above 600,000/µl and often greater than 1 million/µl, which predispose to microthrombosis and microvascular ischemia. Neutrophilia or basophilia may also be seen. A bioassay for thrombopoietin performed at a specialized laboratory can confirm the diagnosis.

Myeloid metaplasia/myelofibrosis (MMM) has been termed *agnogenic myeloid metaplasia, idiopathic myelofibrosis*, or *chronic megakaryocytic-granulocytic myelosis*. This uncommon condition results in intramedullary and extramedullary hematopoiesis that is accompanied by a reactive or secondary marrow fibrosis late in the course of the disease. The hematopoietic precursors most involved are granulocytic and megakaryocytic forms, which infiltrate the spleen and liver. Some cases may be mistaken for AML of megakaryocytic origin. The peripheral blood often has concurrent immature granulocytes and erythroid cells, termed a *leukoerythroblastic reaction*. Erythrocytes may display poikilocytosis with tear-drop formation. Bone marrow aspiration is often difficult, related to the presence of myelofibrosis; therefore core biopsy is recommended to confirm the diagnosis. Survival varies from months to years, depending on the response to treatment for the nonregenerative anemia.

Mast Cell Leukemia and Mastocytemia

Mast cell leukemia may originate in the bone marrow of dogs and is rare. *Mastocytemia* or systemic mastocytosis may occur secondary to a solid mast cell tumor (see Chapter 16) or may suggest severe inflammatory disorders, particularly parvoviral enteritis (Stockham, Basel, and Schmidt, 1986). In general, the larger the number of mast cells in blood, the more likely that systemic mast cell neoplasia is present, especially in the absence of enteritis. Total number of mast cells per blood

smear in dogs with enteritis and mastocytemia usually ranged from 2 to 9, but 30 to 90 mast cells per smear could be found. It is rare to find circulating metastatic mast cells from the mast cell tumors of the skin in dogs and cats. Circulating mast cells are more common in cats affected with the visceral form of mast cell neoplasia producing diffuse, moderate-to-marked splenomegaly. Erythrophagocytosis by the circulating mast cells is not unusual, and this may contribute to anemia. Typically, disseminated disease involves the spleen, liver, distant lymph nodes, or bone marrow. Cytology or surgical biopsy document disseminated mast cell neoplasia best.

Mast cells are not expected in blood smears from healthy dogs (Bookbinder, Butt, and Harvey, 1992). Normal canine marrow has 0 to 1 mast cells/1000 nucleated cells, and greater than 10 mast cells/1000 nucleated cells in bone marrow smears was considered increased and supportive of hemolymphatic involvement in disseminated mast cell neoplasia by O'Keefe and colleagues (1987). Mast cell leukemia can be distinguished from basophilic leukemia based on morphologic and cytochemical criteria.

Malignant Histiocytosis

Malignant histiocytosis or disseminated histiocytic sarcoma is an aggressive multisystem disease with a disseminated neoplasm of myeloid dendritic cells (Affolter and Moore, 2002). Older animals are at greater risk and certain breeds such as golden and flat-coated retrievers, rottweilers, and Bernese Mountain dogs have increase incidence. Primary sites affected include spleen, lung, and bone marrow; secondary sites are liver, lymph nodes, subcutis, and kidney. Anemia, thrombocytopenia, and hyperbilirubinemia are the most common laboratory abnormalities. Cytology demonstrates malignant histiocytes to be large, frequently markedly pleomorphic round or stellate cells with abundant (sometimes vacuolated) basophilic cytoplasm. Nuclei are oval to reniform with lacy chromatin and prominent multiple nucleoli (see Color Plate 6C). Multinucleate cells are common and mitotic figures are often frequent.

Erythrophagocytosis and leukophagocytosis are common but not consistently present. Another condition with erythrophagocytosis and multiple cytopenia is hemophagocytic syndrome of well-differentiated macrophages that may be associated with infectious or inflammatory disease. A more anaplastic appearance of malignant cells and the lack of a history of concurrent infection may distinguish malignant histiocytosis from this condition. Differentiation of malignant histiocytosis from other neoplasms is confirmed by demonstration of positive histiocytic cytochemical and immunohistochemical markers with negative lymphoid or epithelial markers. Similar-appearing cells may actually be T-cell or B-cell lymphoma when immunophenotyping or gene rearrangement studies are performed.

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5

Renee Prater and Harold Tvedten

Hemostatic Abnormalities

The objective of hemostasis is to maintain a delicate balance of coagulation and fibrinolysis to preserve proper vascular structure and function and blood fluidity. When a vascular structure is compromised, it is necessary to have adequate platelet numbers and function, as well as sufficient factors and cofactors of the coagulation system to maintain blood volume and allow a proper environment for wound healing and restoration of vascular integrity. Additionally an intact fibrinolytic system is necessary to localize clotting to the area of vascular damage during the wound-healing process. Disorders of hemostasis are the result of an imbalance in these factors, leading either to hypercoagulation and thromboembolic disorders or to hypocoagulation and hemorrhage. Clinically, hypocoagulability is more commonly observed in veterinary medicine (discussed in detail in the chapter).

The laboratory approach to hemostatic abnormalities is considered in three sections. The first section discusses the components of normal hemostasis, including vascular endothelium, platelets, coagulation factors, and fibrinolysis. The second section presents important technical aspects of selected hemostatic tests. The third section addresses the various inherited and acquired disorders of hemostasis, including references toward breed predisposition and specific diagnostic tests.

Bleeding problems range from overt problems (e.g., severe epistaxis) to suspected problems (e.g., von Willebrand's disease [vWD] in a Doberman pinscher) to asymptomatic problems found during laboratory examination (e.g., decreasing platelet count, evidence of hepatic disease). Signs of bleeding problems may include prolonged bleeding after parturition, estrus, or minor trauma (e.g., venipuncture, loss of deciduous teeth), or spontaneous hemorrhages (i.e., petechiae, ecchymoses, hematomas). Gastrointestinal tract bleeding may appear as fresh red blood or dark tarry stools. Bleeding into joints and other body cavities may be diagnosed from fluid cytology. Small hemorrhages such as petechiae and ecchymoses suggest a platelet or vascular defect (Figure 5-1). Epistaxis is often associated with platelet defects, perhaps because of the paucity of tissue between the vessels and the nasal mucosa. In contrast, coagulation defects are characterized by large, deep hemorrhages (e.g., hematomas, hemarthroses).

Evaluation of a patient with a suspected bleeding disorder relies extensively on performing a complete history and physical examination in addition to the completion of necessary hemostatic tests. A number of screening tests can be run in private practice; however, these tests may not be sensitive enough to identify mild hemostatic disorders. Therefore additional testing by reference laboratories or human hospitals may be necessary to localize the specific hemostatic defect. An organized, simple, and consistent approach should be used to address bleeding problems (Figure 5-2). If no explanation is found for the bleeding or it seems disproportionate to the injury, hemostatic tests are used to document and localize the defect.

Hemostatic defects should initially be localized to a general area of the hemostatic mechanism, which has four main components: the blood vessels, the platelets, coagulation factors, and the fibrinolytic system (Table 5-1). A primary hemostatic plug of aggregated platelets initially seals damaged vessels, after circulating platelets come into contact with exposed subendothelial collagen

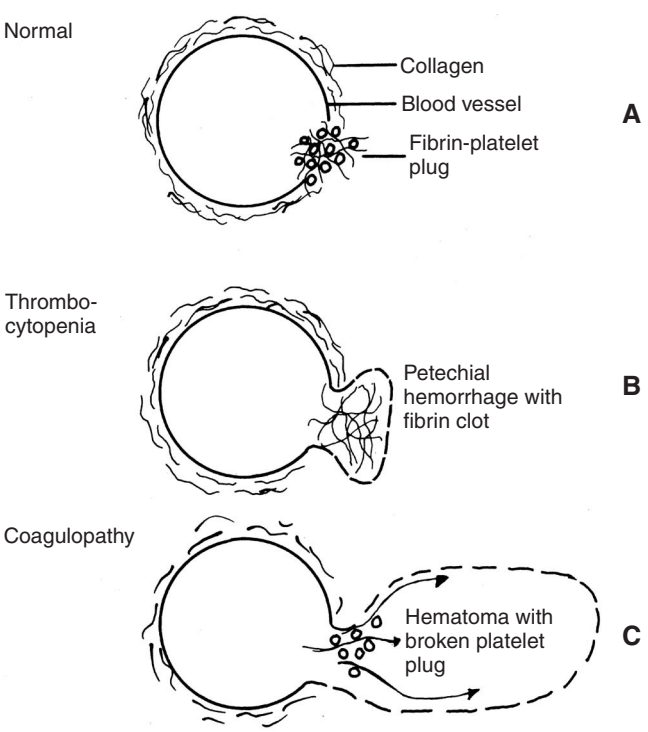

FIGURE 5-1. Physical evidence of thrombocytopenia or coagulopathy. The difference in the size of hemorrhage in thrombocytopenia versus coagulopathy is illustrated. **A,** A break in a vessel is normally repaired quickly when platelets and coagulation factors are stimulated by exposure to collagen to form a platelet plug rapidly stabilized by fibrin strands. **B,** A proper platelet plug is not formed in thrombocytopenia, but only a little blood leaks into the collagen before a fibrin clot is formed. **C,** A coagulation factor defect prevents rapid or firm fibrin stabilization of the platelet plug, and the platelet plug breaks, allowing abundant hemorrhage.

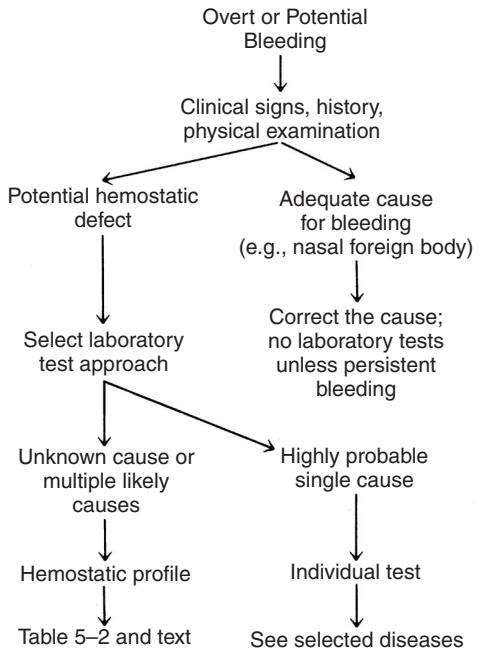

FIGURE 5-2. Selection of laboratory tests. No hemostatic tests are necessary if an adequate explanation for a bleeding process exists. If a hemostatic defect is likely and either several causes are possible or the cause is uncertain, one should screen all parts of the hemostatic mechanism for a defect with a profile of laboratory tests. When the clinical evidence suggests only one probable cause, one should select the most appropriate test to evaluate that defect.

and bind to it with the help of von Willebrand's factor (vWF) (see Figure 5-1; Figure 5-3). The coagulation factors stimulated by the collagen, tissue thromboplastin, and platelets form fibrin strands to stabilize the platelet plug. The fibrinolytic system degrades the clot to reopen the vascular lumen while the vessel heals. A platelet plug forms in coagulopathies, but easily breaks down and allows rebleeding because it is not stabilized by fibrin strands. A severe deficiency of one or more coagulation factors slows the formation of a clot, which should form rapidly after contact with the subendothelial collagen. During this time, extensive hemorrhage can occur before the fibrin clot or pressure of adjacent tissues stops the bleeding (see Figure 5-1).

NOTE: It is usually more effective to use a profile of hemostatic tests during the initial diagnosis of an undefined bleeding problem than to select too few tests to localize the problem.

A profile of five tests is recommended to localize the defect whenever clinically significant bleeding of undetermined cause is seen (see Table 5-1; Table 5-2). Some hemostatic disorders affect multiple areas of the hemostatic mechanism, so evaluation of only one or two tests may lead to an incomplete or erroneous conclusion. Some situations require only a single test (e.g., vWF assay as a presurgical screen for elective surgery on a Doberman dog potentially having vWD; prothrombin time [PT] for suspected ingestion of warfarin-type rodenticide). The recommended profile includes a platelet count, buccal mucosal bleeding time (BMBT) to assess platelet function, activated partial thromboplastin time (APTT) to assess intrinsic and common coagulation

TABLE 5-1. Hemostatic Mechanisms: Functions and Appropriate Laboratory Tests

	PLATELET NUMBERS*	**PLATELET FUNCTION***	**COAGULATION FACTORS***	**FIBRINOLYTIC SYSTEM***	**BLOOD VESSELS**
Normal function†	Platelet plug	Platelet plug	Fibrin clot	Dissolve clot	Retain blood
Tests for evaluation‡	Platelet count	Bleeding time	APTT or ACT	Plasmin activity	Vascular integrity
	Platelet estimate	(BMBT)	PT Specific Factor Analysis	FDP	Histopathology
Term for disease	*Thrombocytopenia thrombopathy*	*Platelet dysfunction,*	*Coagulopathy*	*DIC*	Vascular disease

*Five basic portions of the hemostatic mechanism.
†Basic function of the five portions. All portions interact with the others (e.g., the fibrin clot stabilizes the platelet plug, clots stimulate fibrinolysis, platelets accelerate the clotting process).
‡The hemostatic parameter in bold type is evaluated by these tests. Possible abnormality that may be identified from the tests is italicized. Coagulation factor activity interpretation is described in the text and in Figures 5-4, 5-7, 5-8, 5-10, and 5-11.
BMBT, Buccal mucosal bleeding time; *APTT,* activated partial thromboplastin time; *ACT,* activated coagulation time; *PT,* prothrombin time; *FDP,* fibrin degradation products; *DIC,* disseminated intravascular coagulation.

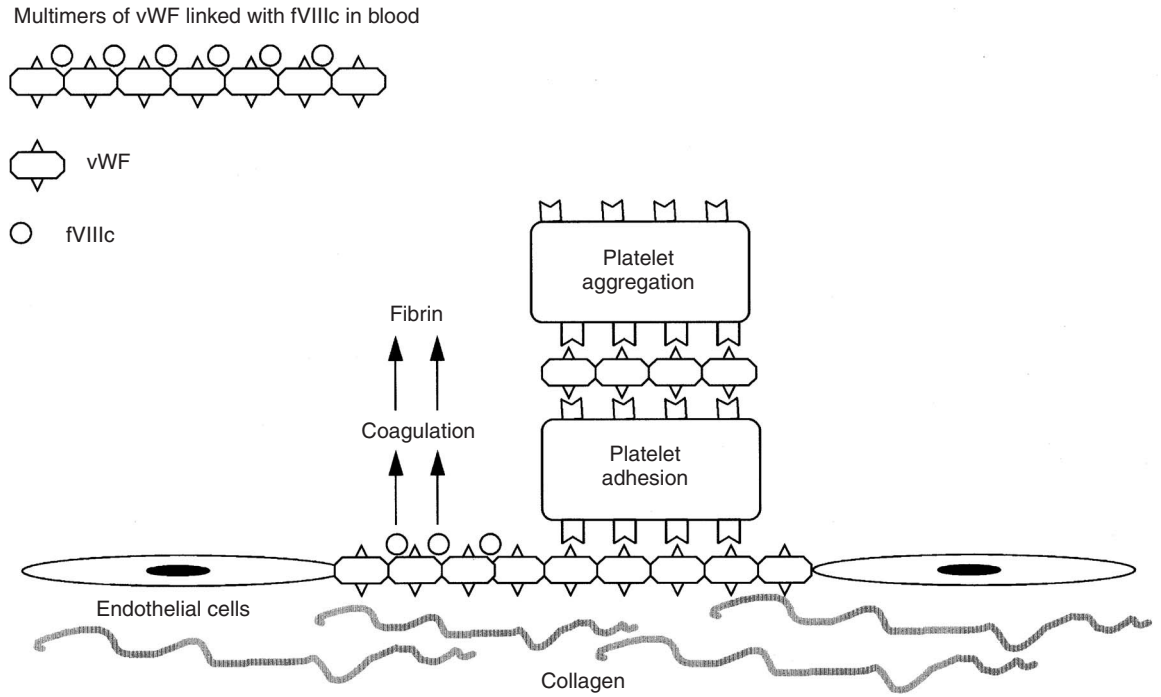

FIGURE 5-3. Depiction of the factor VIII molecular complex. von Willebrand's factor *(vWF)* is a long multimer of many vWF units. Factor VIII coagulant activity *(fVIIIc)* is a smaller molecule linked to vWF in the complex that protects fVIIIc. To facilitate platelet adhesion to sites of vascular injury, vWF binds to collagen at the sites; vWF also facilitates receptor specific platelet aggregation. In the intrinsic system, fVIIIc acts to activate the common pathway at factor X, which ultimately results in fibrin formation to stabilize the platelet plug. (Modified from Cotran RS, Kumar V, Robbins SL: *Robbins pathologic basis of disease*, ed 5, Philadelphia, 1994, WB Saunders, p 621.)

pathways, PT to assess extrinsic and common pathways, and assay of fibrin degradation products (FDP) to screen for fibrinolytic activity (see Table 5-2). Alternative tests are substituted if required. Activated coagulation time (ACT) is a substitute for the APTT; it is simpler to perform but less sensitive and specific than the APTT. Estimation of platelet numbers on a blood film is often adequate to detect clinically significant thrombocytopenia.

COMPONENTS OF NORMAL HEMOSTASIS

Vascular Endothelium

Healthy vascular endothelial cells secrete substances such as prostacyclin and nitric oxide that act in an antithrombotic manner as vasodilators and inhibitors of platelet adhesion. However, when the vascular endothelium is disrupted because of trauma, inflammation, neoplasia, or toxicity, coagulation is initiated by platelet adherence to

TABLE 5-2. Hemostatic Screening Profile

PREFERRED TEST	ALTERNATIVE TEST	PARAMETER TESTED
Platelet count	Blood smear	Platelet quantity
BMBT	Clot retraction	Platelet function
APTT	ACT	Coagulation factors
PT	None	Coagulation factors
FDP	None	Fibrinolysis
vWf assay	Botrocetin cofactor	vWF

BMBT, Buccal mucosal bleeding time; *APTT,* activated partial thromboplastin time; *PT,* prothrombin time; *FDP,* fibrin degradation products; *ACT,* activated coagulation time; *vWF,* von Willebrand's factor.

exposed subendothelial collagen, which initiates the coagulation cascade and the formation of a fibrin mesh that subsequently stabilizes the clot until repair can be affected. Clot retraction caused by thromboxane A_2-mediated vasoconstriction also occurs, which further guards against hemorrhage and continued blood loss.

Platelets

Platelets are small cytoplasmic fragments from megakaryocytes; they circulate for approximately 5 days in dogs and about 30 hours in cats. The spleen sequesters approximately one fourth to one third of the total circulating platelets, so splenic contraction from an excitement (i.e., epinephrine) response results in a pseudothrombocytosis. Platelets are released into the blood in proportion to stimulation from thrombopoietin; circulating thrombopoietin concentration is controlled by the total circulating platelet mass, not absolute platelet number; therefore there may be mild thrombocytopenia with macroplatelets. Cavalier King Charles spaniels have a breed-related dysplasia with large platelets, low platelet numbers, and probably normal platelet mass.

Platelets are the predominant players in primary hemostasis, and they are also instrumental in secondary hemostasis by providing soluble factors for clot stabilization. Platelets contain several distinct types of granules, including alpha, dense, and lysosomal bodies. Alpha granules contain adhesive factors that promote platelet aggregation and adhesion to subendothelium (i.e., vWF, fibronectin, fibrinogen, thrombospondin, vitronectin, P-selectin), factors that stimulate vascular repair (i.e., platelet-derived growth factor, transforming growth factor beta, platelet factor 3 [PF-3], connective tissue–activating peptide III, high molecular weight kininogen [HMWK]), and coagulation factors (i.e., factor V, factor XI, plasminogen activator inhibitor I, protein S). Dense granules contain nucleotides and amines including adenosine triphosphate (ATP), guanosine triphosphate (GTP), adenosine diphosphate (ADP), guanosine diphosphate (GDP), calcium, seratonin, and pyrophosphate. Lysosomal bodies contain an assortment of soluble acid-dependent hydrolases involved in intracellular protein degradation.

Platelets adhere to exposed subendothelial collagen with the help of vWF after vascular injury. This results in a temporary hemostatic plug, termed *primary hemostasis*. This binding of platelets results in a conformational change to expose previously internalized phosphatidylserine (formerly known as PF-3, a potent receptor for factor X and prothrombin), which causes platelet activation, release of platelet granule contents, aggregation of more platelets, and initiation of secondary hemostasis by formation of a procoagulant surface of fibrinogen receptors on the primary plug. Release of ADP from the dense granules causes synthesis of thromboxane A_2, a prostaglandin that causes irreversible platelet aggregation and viscous metamorphosis and local vasoconstriction.

Coagulation Factors

Secondary hemostasis occurs after formation of the initial platelet hemostatic plug over an area of vascular injury. This is a process of stabilization of this plug by a mesh of fibrin formed in the coagulation cascade, and it is especially important where blood flow and pressure are greater. The primary objective of the coagulation system is the formation of thrombin, which converts circulating fibrinogen to insoluble fibrin. These generated fibrin strands then cross-link to form a mesh, which traps more blood cells, stabilizes the platelet plug, and provides more effective hemostasis at the site of vascular compromise; the fibrin plug is stronger than the primary plug and is resistant to proteolytic degradation by plasmin, a fibrinolytic enzyme.

The coagulation mechanism is a cascade of proenzymes that require activation to function, and can be divided into three parts: (1) intrinsic pathway, (2) extrinsic pathway, and (3) common pathway. A simplified version of the coagulation cascade for diagnosis is diagrammed as a letter *Y* (Figure 5-4). Several clotting factors (specifically II, VII, IX, and X) are dependent on vitamin K to be active; chemicals that act as vitamin K antagonists (i.e., anticoagulant rodenticides) interfere with coagulation by interfering with this activation mechanism. In the case of a difficult venipuncture, tissue thromboplastin may be released from injured cells and initiate the clotting cascade ex vivo. Deficiencies of tissue thromboplastin and ionized calcium levels are never low enough in vivo to cause bleeding.

The intrinsic pathway includes factors XII, XI, IX, and VIII and is initiated by contact activation, where circulating factor XII contacts a nonendothelial surface such as collagen outside a blood vessel. Activated platelets are

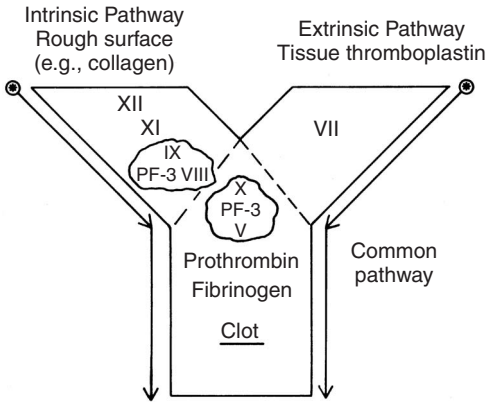

FIGURE 5-4. A simplified coagulation cascade. One may consider the cascade as a letter *Y* with three portions (or pathways): The intrinsic pathway includes factors XII, XI, IX, and VIII; the extrinsic pathway contains factor VII; and the common pathway includes factors X, V, prothrombin, and fibrinogen. Platelet factor 3 (i.e., phosphatidylserine) on the surface of activated platelets speeds the coagulation process. The intrinsic pathway is initiated by contacting abnormal surfaces (e.g., collagen) not covered by endothelium. The intrinsic system activates the common pathway at factor X by a complex of IX, VIII, and platelet factor 3 (PF-3). The end point of the common pathway (and thus the intrinsic or extrinsic pathways) is the fibrin clot. Tissue thromboplastin from damaged cells and factor VII in the extrinsic pathway also activates the common pathway.

important in clot formation, because they provide a negatively charged phospholipid surface on which coagulation factors assemble. In severe thrombocytopenia, the coagulation process is slower (e.g., slight prolongation of ACT). The negatively charged surface of platelets reacts with factors IX and VIII as a complex in the intrinsic pathway. Activated factor IX from this complex interacts with another complex of factors X and V of the common pathway.

The extrinsic pathway is initiated by interaction of tissue factor and activated factor VII, and is considered to be more important clinically, as activated factor VII activates both factors IX in the intrinsic pathway and X in the common pathway. The extrinsic system initiates the coagulation response, whereas the intrinsic system sustains the generation of fibrin. These two pathways converge into the common pathway at the point where factor X is activated, which then leads to a fibrin clot formation. The common pathway consists of factors X, V, prothrombin, and fibrinogen. Once coagulation enzyme factors have been activated, they are inhibited by antithrombin III complexed with heparin and removed by the phagocytic system. Those that escape antithrombin III will bind with thrombomodulin on endothelial cells, which activates protein C. Protein C is an anticoagulant and profibrinolytic factor which, with protein S as a cofactor, specifically hydrolyses factors V and VIII and inhibits thrombin formation (Mosnier, Meijers, and Bouma, 2001).

Heparin inhibits thrombin and other procoagulant factors indirectly by activating ATIII, a circulating natural anticoagulant that is the main physiologic inhibitor of thrombin. Because ATIII is usually decreased in disseminated intravascular coagulation (DIC), heparin therapy may be ineffective, although it has been used clinically to treat hemostatic abnormalities associated with severe gastrointestinal disease, septicemia, and endotoxemia (Moore and Hinchcliff, 1994). In people with ATIII concentrations of less than 40% of normal, the response to heparin is poor. Decreased ATIII occurs in hepatic insufficiency, owing to decreased synthesis, and in glomerular disease because of increased loss with proteinuria. Protein-losing enteropathies may also decrease ATIII. Availability of the ATIII assay varies. Automated assays with chromogenic substrates are used by some veterinary laboratories and allow routine testing. However, tests of ATIII function are expensive in terms of time and reagents to maintain, so they are available only at a few hemostasis reference laboratories. Knowledge of the activity and amount of these anticoagulants and ATIII in various diseases or in specific patients is needed to fully predict the outcome of a thrombotic episode.

Fibrinolysis

Fibrinolysis is the process of fibrin clot dissolution once the damaged blood vessel is repaired, which reestablishes normal vascular patency. The enzyme plasmin digests circulating fibrinogen and insoluble fibrin clots and produces FDP or fibrinogen degradation products (FgDP), which have anticoagulant effects. Measurement of fibrinolysis can include FDP quantification or measurement of the proteolytic fragment of fibrinolysis, D-dimer.

LABORATORY TESTS

When a profile of tests is used, one should interpret each test individually and list the individual conclusions of what is normal or abnormal. Then one makes a disease diagnosis based on the laboratory pattern and

TABLE 5-3. Expected Hemostatic Test Results in Selected Diseases

	HEMOSTATIC PROFILE				
DISEASE	BMBT	PLATELET COUNT	APTT	PT	FDP
Thrombocytopenia (e.g., ehrlichiosis)	I	D	N	N	N
Platelet dysfunction (e.g., aspirin treatment)	I	N	I	N	N
Intrinsic pathway defect (e.g., hemophilia A or B)	N*	N	I	N	N
Factor VII deficiency	N	N	N	I	N
Multiple factor defects (e.g., vitamin K antagonism)	N*	N	I	I	N
Common pathway defect (e.g., factor × deficiency)	N*	N	I	I	N
Disseminated intravascular coagulation (DIC)	I	D	I	I	I
von Willebrand's disease	I	N†	N‡	N	N

*Initially stops in normal time period but may start bleeding again.
†Mild thrombocytopenia may occur if patient is concurrently hypothyroid.
‡Usually normal but may be increased.
BMBT, Buccal mucosal bleeding time; *APTT,* activated partial thromboplastin time; *PT,* prothrombin time; *FDP,* fibrin degradation products; *I,* increased; *D,* decreased; *N,* normal. See text for details on these diseases and others that may give similar patterns.
DIC can have widely variable results (see Table 5-6).

clinical evidence. (The reader should see Table 5-3 for typical patterns expected in various diseases and refer to it when diseases in the next section are discussed.) Proper sample collection and management are important for accurate diagnosis of a suspected hemostatic disorder. Clean venipuncture is essential to minimize introduction of tissue factor (i.e., tissue thromboplastin), which could falsely activate platelets and clotting factors. Additionally, turbulence, clotting, or extremes in temperature may invalidate hemostatic testing.

Blood Vessel Evaluation

Although listed as the first type of test, vascular disease is rarely diagnosed as the cause of bleeding in dogs and cats. Blood vessels can most effectively be evaluated histologically. Skin biopsy may document vasculitis in bleeding animals, and is indicated in the presence of edema, petechiae, and a normal platelet count, or edema plus DIC. Immune-mediated vasculitis in dogs is uncommon but antigen-antibody complexes occasionally deposit in vessels, causing an Arthus reaction and nonthrombocytopenic purpura with petechiae and edema. Rare examples of vascular disease in small animals include connective tissue diseases such as Ehlers-Danlos syndrome or feline epitheliogenesis imperfecta. In rare instances, cutaneous hemorrhages are noted in dogs with hyperadrenocorticism. The bleeding is secondary to catabolism of supporting collagen around vessels.

Platelet Estimate

Thrombocytopenia is a common cause of bleeding; an estimate from a blood smear is a quick, inexpensive, and effective means of diagnosis. After routine preparation and staining of a blood smear, one should determine the average number of platelets in 5 to 10 microscope fields using the 100x oil objective (i.e., 1000x magnification) to estimate platelet numbers (Tvedten, Grabski, and Frame, 1988). This should be adequate to rank the count as very low, low, normal, or high (Figure 5-5). Dogs normally have 8 to 29 platelets/field, and cats should have 10 to 29/field. If the thrombocytopenia is severe enough to cause bleeding, then one sees only 0 to 3 platelets/field, so the difference is easy to detect. One should only count platelets in the thin area where one properly evaluates leukocyte morphology. On most smears, this is where the red blood cells (RBCs) infrequently touch each other and central pallor in canine RBCs is prominent. Anemic, bleeding animals have fewer RBCs per field in the proper area. An alternative is to consider 1 platelet/100x oil immersion field as approximately equal to 15,000 platelets/μl. One must ensure uniform platelet distribution. If platelet clumps are present, neither the platelet count nor the estimate may be trusted. Platelet clumps that are large are usually pulled to the distal end of the smear and are large enough to be seen at scanning magnification (10x).

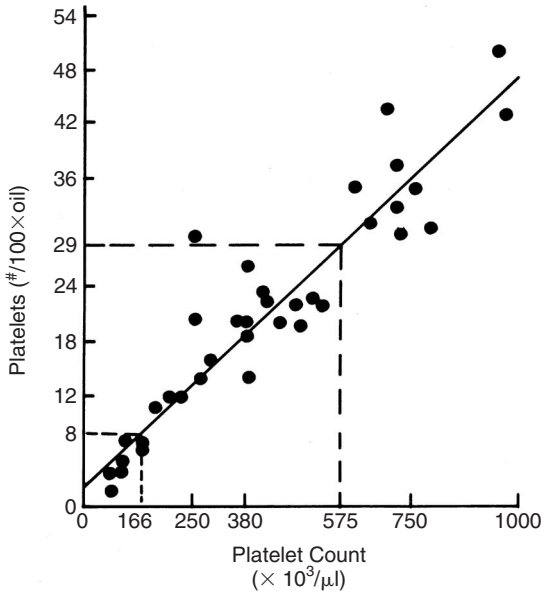

FIGURE 5-5. Estimation of platelet numbers for canine blood smears. The number of platelets in an average 100x oil immersion field may be used to estimate the platelet count. Between 8 and 29 platelets/100x oil immersion field represents a normal canine platelet count. A *regression line* indicates the relationship of the two factors.

NOTE: Thrombocytopenia is a very common cause of bleeding and an estimate from a blood smear is a quick, inexpensive, and effective means of diagnosis.

Platelet Count

Platelet counts are useful to classify the severity of thrombocytopenia and monitor the course and response of the disease to treatment. Platelet numbers in normal dogs are approximately 200 to 700×10^9/L (200 to 700×10^3/μl) and healthy cats have 300 to 800×10^9/L (300 to 800×10^3/μl) (see discussion of total cell counts in Chapter 2 and Appendix II). Collection and handling are especially important. A venipuncture adequate for a routine CBC is often inadequate for an accurate platelet count. A two-syringe or two-Vacutainer technique is recommended in cases of suspected thrombocytopenia. The first blood out of a needle contaminates the container with tissue thromboplastin and stimulates platelet clumping. One should remove the first syringe or Vacutainer containing the tissue fluids and collect a sample in a second Vacutainer or syringe. Allowing blood to flow from a bare needle also flushes out tissue thromboplastins. Ethylenediaminetetraacetic acid (EDTA) is an excellent anticoagulant for canine platelet counts, though exceptions occur in which EDTA (or other anticoagulants) seem to promote clumping of platelets (or white blood cells [WBCs]) to cause pseudothrombocytopenia.

Feline platelets tend to clump, frequently causing inaccurate platelet counts. If platelet clumps are visible, a vortex mixture can be used to shake the blood before repeating the platelet count. Often the mixing results in the platelet count increasing into the normal reference range, ruling out thrombocytopenia. Other errors in the platelet count include unusually large platelets (especially in cats) that exceed an instrument's range for counting and thus are excluded. In feline myeloproliferative disorders, many large and bizarre platelets may not be counted. Small particles (e.g., lipid droplets, RBC fragments, ghost cells) may be counted as platelets in lipemic blood or in intravascular hemolytic anemia. An abnormal appearance of platelet histograms or cytograms suggests this error. Small RBCs in iron deficiency anemia can be counted as platelets.

One may use manual counts with a hemocytometer or counts from automated hematology cell counters. Automated platelet counts are more precise, easier, and usually more accurate. The hemocytometer must be clean and free of scratches. Because platelets are the size of dust particles, nicks and scratches in the glass may cause misidentification of dust as platelets. One should keep one very good hemocytometer just for platelet counts. A phase contrast microscope helps to differentiate platelets from other particles. Lowering the condenser on a regular microscope also aids in visualizing the relatively transparent platelets. A platelet stain improves platelet identification in a hemocytometer but is usually unnecessary.

Delayed analysis allows platelet clumping and can lead to a lower platelet count. Various references suggest 30 minutes to 6 hours as a maximum tolerable limit, but an evaluation of 21 dogs with the Bayer H-1 hematology analyzer suggested that clinically accurate platelet counts could be obtained with analysis of EDTA blood up to 2 days old (Figure 5-6). This suggests that EDTA blood sent in the mail for 1 or 2 days may still have clinically acceptable platelet count results if platelet clumping is absent or minimal.

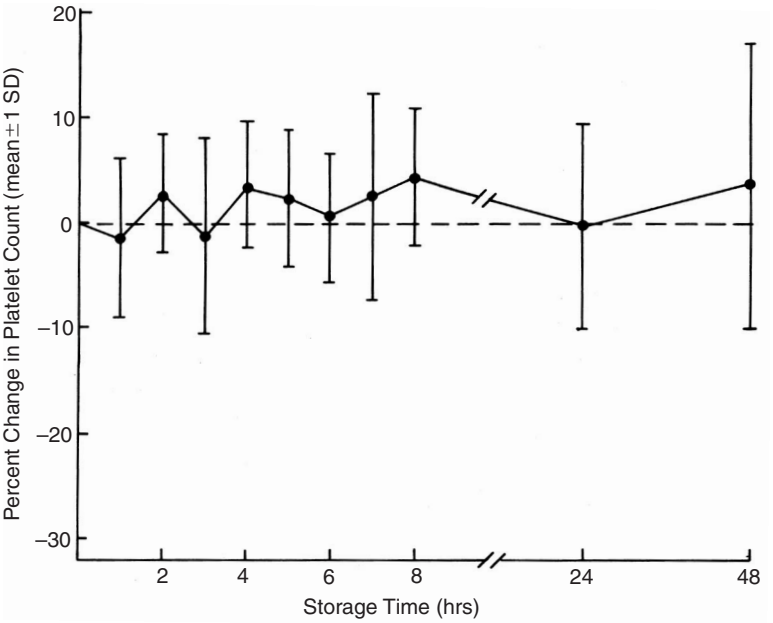

FIGURE 5-6. Stability of the platelet count. The mean percentage change in the platelet count with time is compared with the initial platelet count for 21 canine ethylenediaminetetraacetic acid *(EDTA)* blood samples stored at room temperature for 8 hours and refrigerated for 2 days. *Bars* indicate the standard deviation *(SD)*.

Platelet Morphology

When reviewing blood films for platelet numbers and clumping, one should note platelet size and morphology. Numerous large platelets suggest active thrombopoiesis and an active bone marrow. "Increased numbers of large platelets" is a subjective statement, but mean platelet volume (MPV) and platelet histogram objectively describe changes in platelet size. Larger platelets are often seen in thrombocytopenia, and are more functionally active, which may explain why some dogs with platelet counts of less than 10,000/µl do not bleed. Canine MPV may also be artificially high in EDTA blood samples held more than 4 hours at 4° C because of platelet swelling. Microplatelets may occur in iron deficiency anemia, bone marrow aplasia, and immune-mediated thrombocytopenia (ITP). Platelets occasionally have pseudopodia and an irregular shape that may be related to platelet activation during collection and handling of the sample. Primary platelet disorders such as thrombasthenic thrombopathia in otter hounds, foxhounds, and Scottish terriers are rare causes of large and morphologically bizarre platelets. *Ehrlichia platys* may be visible in platelets.

MPV, platelet mass, and platelet distribution width (PDW) are available from some hematology analyzer systems. The average MPV is 6 to 9 fl in dogs and 14 to 18 fl in cats. Increased MPV usually indicates increased megakaryocytic shedding of platelets. Decreased MPV noted with the Bayer H-1 analyzer in thrombocytopenic dogs has been associated with ITP. Decreased MPV may also occur if small particles (e.g., lipid droplets, RBC fragments) are counted as platelets. Platelet mass is the product of platelet number times MPV. Platelet mass is a more physiologic measurement. For example, Cavalier King Charles spaniels have often a low platelet count, yet have large platelets and a high enough MPV to have a platelet mass similar to other breeds. Increased PDW indicates increased variation in platelet volume.

Platelet Function Testing

Platelet function tests are typically done when a defect in primary hemostasis is suspected but no evidence for thrombocytopenia, vWD, or history of aspirin exposure is found. These include platelet aggregation, BMBT, clot retraction, and glass bead retention test. Platelet aggregation test measures the ability of platelets to stick together and release granule contents, as measured by a platelet aggregometer by optical or impedance techniques.

NOTE: The BMBT is a very useful, in-clinic screening test for thrombocytopenia or reduced platelet function. BMBT detects vWD and can be used as a presurgical test.

It is a specialized technique and not usually available for clinical diagnosis.

Buccal Mucosal Bleeding Time

The BMBT is a sensitive and specific platelet function test in dogs, and it is typically used as a presurgical screen to rule out defects in primary hemostasis. A spring-loaded disposable device creates standardized cuts in the mucosal surface of the upper lip, which has been everted and tied tightly enough to cause vascular congestion by impeding venous return. The technique involves measuring the time for bleeding to cease. Blood is carefully removed by blotting near the incision with filter paper, without applying pressure to the wound. The BMBT in healthy dogs is approximately 2.61 ± 0.48 minutes and can be used to diagnose severe thrombocytopenia, vWD, and uremia. BMBT may also be helpful in diagnosing clotting abnormalities associated with DIC. The BMT test is much more sensitive and consistent than the cuticle-bleeding test. A cuticle-bleeding time (CBT) is simply performed by clipping the toenails just short enough to get bleeding and observing when the bleeding stops. It should stop within 5 minutes. The advantage is that this can be a routine part of presurgical preparation of the patient. Clinical experience suggests that the CBT is inconsistent (e.g., one dog had a platelet count of 22,000/µl, yet a normal CBT). The BMBT is usually "normal" in coagulation defects such as hemophilia, because the bleeding stops initially in the expected time because of the formation of platelet plugs. However, because fibrin strands do not stabilize the platelet plug, the incision is prone to rebleed.

Clot Retraction Test

This crude, insensitive test of platelet function is not recommended. After a tube of blood has clotted, it is kept at 37° C. Inspection after 1 hour should reveal serum squeezed out of the clot by the contraction of platelets and thus visible serum between the clot and the wall of the tube. Abnormal lysis (e.g., clot lysis during a 24-hour incubation at 37° C) suggests increased fibrinolytic activity.

Glass Bead Retention Test

The platelet glass bead retention test is useful in detecting general abnormalities in hemostasis, including platelet adhesion defects in thrombopathias and vWD (Brassard and Meyers, 1991). After platelet numbers have been determined, then a standard volume of venous blood is passed at a standard rate through a plastic column packed with glass beads. Retention is dependent upon vWF, ADP release from hemolyzed RBC, normal platelet function, and coagulation.

Automated Platelet Activation Assay

An automated monitoring assay of platelet activation in blood (collected in EDTA and citrate), theophylline, adenosine, and dipyridamole tubes) stored at 4° C and evaluated within 60 to 180 minutes of collection may be useful for evaluating platelet activation in animals.

NOTE: The ACT Vacutainer tube test is a simple, inexpensive screening test to evaluate the intrinsic and common coagulation system (e.g., warfarin toxicity).

Activated Coagulation Time

The ACT is a simple, inexpensive screening test to evaluate the intrinsic and common coagulation system. It is insensitive. A specific coagulation factor deficiency must be less than 5% to increase the ACT. (The reader should note that the routine ACT or APTT would not detect a carrier of hemophilia with 40% to 60% of factor VIII or IX.) The ACT may be slightly prolonged in thrombocytopenia. ACT reference values are approximately 60 to 110 seconds for dogs and 50 to 75 seconds for cats. It is a whole blood procedure, obviating the need for using anticoagulants or obtaining plasma. Only a special ACT Vacutainer tube that contains an activator is needed.

One should use a two-tube collection in which the first tube containing any tissue thromboplastin from the venipuncture is discarded or used for another purpose. Special care should be taken to obtain a clean sample to avoid contamination of the sample with tissue factor, which would activate the extrinsic pathway. A heating block or water bath is used to prewarm the tubes to 37° C and maintain that temperature until the clot forms. The blood is incubated at 37° C for 60 seconds after blood enters the tube for a canine sample, 45 seconds for a feline sample. Then, at 5-second intervals, the tube is inverted until the end point of the first visible clot is detected.

The time from addition of blood to the tube until a clot first becomes visible is the ACT.

Prothrombin Time

The PT evaluates the extrinsic and common pathways (Figure 5-7). The factors in the common pathway include X, V, II, thrombin, and fibrinogen. One of the major uses of the PT, as single test, is detection of vitamin K antagonist poisoning (e.g., warfarin) or other situations in which hepatic synthesis of vitamin K-related coagulation factors is impaired. PT is the most sensitive test of warfarin-type toxicity. Of the vitamin K-related factors (II, VII, IX, and X), factor VII has the shortest half-life. If synthesis of these factors is inhibited, factor VII deficiency develops earliest. Because factor VII is in the extrinsic pathway, the PT is prolonged earliest and has the greatest relative increase above the normal mean. The PT is used in conjunction with the APTT or ACT (Figure 5-8) in other types of coagulopathies.

NOTE: PT is the most sensitive test of warfarin-type toxicity.

The citrated plasma-clotting tests (i.e., APTT, PT, Fibrinogen, thrombin time [TT], FDP) require nine parts fresh whole blood and one part 3.8% trisodium citrate anticoagulant to separate plasma from cells and platelets within 30 minutes of sample collection. Citrate or oxalate blood collection tubes must be filled completely to a mark on the label to avoid errors in dilution. Plasma can be stored at 4° C for 48 hours, 20° C for 6 hours, or frozen for transport to preserve factors VII and VIII. Calcium and activating agents are added during the tests, and fibrometers or photooptical coagulometers are used to detect formation of fibrin strands or changes in the intensity of filtered light when clot formation occurs. PT measures the time required for fibrin clot formation after addition of tissue thromboplastin and calcium. PT is increased with factor deficiencies in the extrinsic or common system, DIC, acquired vitamin K deficiency such as in coumarin poisoning, bile insufficiency, or liver failure.

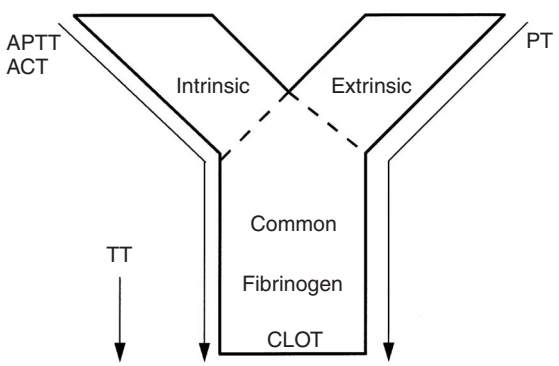

FIGURE 5-7. Hemostatic tests of the coagulation cascade. The activated partial thromboplastin time *(APTT)* and activated coagulation time *(ACT)* test all factors of the intrinsic and common pathways. The modified *TT* determines the fibrinogen concentration. The prothrombin time *(PT)* tests factor VII and the factors of the common pathway.

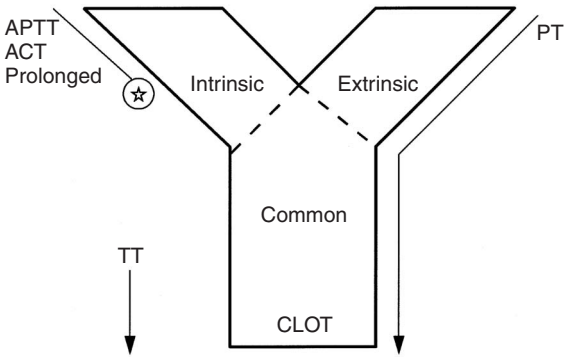

FIGURE 5-8. Test results for an intrinsic pathway defect. Only activated partial thromboplastin time *(APTT)* and activated coagulation time *(ACT)* results should be abnormal. A *star and termination of the arrow* along side of the intrinsic system depict the site of the defect. Specific factor analysis and consideration of clinical signs, history, and physical findings identify a specific factor defect from factors XII, XI, IX, and VIII.

Activated Partial Thromboplastin Time

The APTT is the most sensitive test of the intrinsic pathway (i.e., factors XII, XI, IX, VIII) plus the common pathway factors (i.e., all factors except VII; see Figure 5-7). It is used to detect decreased activity of one or more coagulation factors, as in hemophilia, vWD, DIC, acquired vitamin K deficiency, coumarin poisoning, bile insufficiency, or liver failure. APTT measures the time required for fibrin clot formation after addition of a contact surface activator of the intrinsic system and calcium.

NOTE: The APTT is the most sensitive and specific test of coagulation factors.

Factors must be less than 30% of normal to prolong the APTT.

Thrombin Time

TT measures concentration of normal fibrinogen (see Figure 5-7). TT may be used to monitor the anticoagulant activity of heparin and FDP. The modified TT has an excess of thrombin added to the reagent, making it insensitive to anticoagulants. The modified TT is the type usually used in veterinary laboratories and should not be used to monitor heparin treatment. Heat precipitation methods to measure fibrinogen concentration, which are typically used in the diagnosis of hyperfibrinogenemia associated with inflammatory disease, are not sufficiently sensitive to diagnose hypofibrinogenemia. Hypofibrinogenemia is primarily the result of increased consumption in DIC, but decreased production of fibrinogen may also occur in advanced liver disease.

Specific Factor Analysis

Specific factor assays are performed when a hemostatic defect has been localized to a likely factor or group of factors (e.g., early intrinsic pathway factors). Hemostasis laboratories use a modification of the APTT to determine the percentage of specific factors (e.g., percentage of factor VIII in hemophilia A). The percentage is compared with normal pooled plasma, so about 100% is normal.

Immunologic assays (i.e., electroimmunoassay or enzyme-linked immunosorbent assay [ELISA]) of vWF are used to diagnose vWD, and several subtypes of vWD have been identified by precise characterization of the size and concentration of multimers of the molecule (Thomas, 1996). The incidence of vWD in some breeds (e.g., Doberman pinschers, Airedale terriers) may be 50% or more, so any bleeding tendency or need for surgery is a reasonable indication for vWF assay. One should measure vWF before any blood transfusions are administered, because transfusions add vWF (which can mask a deficiency in vWD); vWF has also been shown to fluctuate with thyroxine concentrations, with systemic illness, stress, strenuous exercise, azotemia, liver disease, parturition, and after administration of the vasopressin analogue desamino-D-anginine vaporessin (DDAVP). Canine-specific vWF antigen assay has been validated in both cats and dogs to diagnose vWD. VWF function assays (e.g., botrocetin) can also be performed.

Shipping plasma to referral laboratories requires special care. Laboratories should be asked about the required handling procedures; typically the plasma can be stored at 4° to 22° C for up to 48 hours or rapidly frozen in 1 ml aliquots and shipped on dry ice. One should use 3.8% citrate as the anticoagulant in a 1 : 9 ratio (e.g., 0.5 ml Na citrate + 4.5 ml blood), keep the sample refrigerated or on ice, and separate the plasma within 30 minutes of collection by high-speed centrifugation (i.e., 2500 to 3500 rpm) for 15 minutes. Plasma should be collected using plastic pipettes and containers.

Fibrin Degradation Products

FDP assays are primarily used to document an increased breakdown of fibrin clots in the body. This is usually related to intravascular clotting in the body and suggests DIC, which is a common acquired disorder secondary to a variety of inflammatory, neoplastic, or necrotic diseases. A latex agglutination test that uses antihuman FDP with cross-reactivity to feline and canine FDP was commonly used in veterinary laboratories. It is being replaced by other tests of FDP, such as the plasma FDP test. Specific assays of D-dimer concentrations appear to be sensitive and specific ancillary methods to support a diagnosis of DIC in dogs. D-dimer is the proteolytic fragment of fibrinogen degradation (Stokol et al, 1999 and 2000).

DISORDERS OF HEMOSTASIS

Disorders of Vascular Endothelium

Inherited Vascular Wall Disease

von Willebrand's Disease • VWD is the most common inherited hemostatic defect that has been reported in many breeds of dogs, including Doberman pinschers, Scottish terriers, Shetland sheepdogs, and Chesapeake Bay retrievers (Brooks, Dodds, and Raymond, 1992; Venta et al, 2000). vWF is required for adhesion of platelets to subendothelium at sites of vascular damage and to other platelets (see Figure 5-3). Another function of vWF is to bind with factor VIII to stabilize

NOTE: VWD is the most common inherited hemostatic defect and it is usually detected by the BMBT and vWF assays.

the molecule and prevent rapid clearance from the circulation, so a differential diagnosis in a suspected vWD patient is hemophilia A (Budde et al, 2002).

The clinical signs of vWD are often mild and variable. Often owners and surgeons are unaware of any bleeding tendency in affected dogs until a surgical procedure is performed. With the exception of the BMBT and vWF assay, most of the common hemostatic tests (i.e., APTT, ACT) fail to identify vWD despite very low levels of vWF. Predisposition to bleeding tends to be noted with vWF levels of 30% or less, and bleeding tends to be greater with worsening deficiency of vWF. Type I vWD is characterized by decreased vWF antigen with proportional decreases in all multimers, and it has been reported in many canine breeds. Type II vWD has decreased vWF antigen, but only the largest multimer has diminished concentrations; it has been reported in German shorthaired and wirehaired pointers. Type III vWD has essentially no vWF in the homozygotes and has the most severe clinical signs; it has been reported in Scottish terriers, Shetland sheepdogs, and Chesapeake Bay retrievers. vWD is differentiated from hemophilia A by vWF concentration (Table 5-4); vWF is normal to increased in hemophilia A. The APTT is consistently increased in hemophilia A, whereas in vWD the APTT is often normal despite the deficiency of vWF. A transfusion transiently raises vWF levels and can confuse test results. Treatment recommendations include administration fresh frozen plasma (or cryoprecipitate) for the treatment or prophylaxis of hemorrhagic episodes in dogs with vWD or hemophilia (Stokol and Parry, 1998).

Ehlers-Danlos Syndrome • Several, rare inherited disorders of the vasculature result in aberrant hemostasis. Cutaneous asthenia or Ehlers-Danloslike syndrome is one disorder that occurs primarily in dogs and cats (Sinke, van Dijk, and Willemse, 1997). Cutaneous asthenia, or dermatosparaxis, is an inherited disease of abnormal collagen synthesis that results in increased vascular fragility, joint laxity, and hyperextensibility of the skin. Skin tears are accompanied by hemorrhage and defects in platelet function that have been managed successfully with administration of desmopressin (i.e., DDAVP).

TABLE 5-4. Differentiation of von Willebrand's Disease and Hemophilia A

TEST	VON WILLEBRAND'S DISEASE (vWD)	HEMOPHILIA A
APTT	Usually normal	Increased
BMBT	Prolonged	"Normal"
VWF	Decreased	Normal to increased

APTT, Activated partial thromboplastin time; *BMBT,* buccal mucosal bleeding time; *vWF,* von Willebrand's factor.

Familial Vasculopathy • A second inherited vascular wall disease is familial vasculopathy, which is a rare idiopathic inherited vasculopathy that results in vasculitis and collagenolysis. This disorder has been reported in several canine breeds, including the German shepherd, beagle, Scottish terrier, and greyhound (Weir et al, 1994). Focal collagen degeneration, vasculitis, swelling, and high PF-3 levels characterize this disorder. Phosphatidylserine, or PF-3, is a platelet membrane component that plays an important role in the activation of the coagulation mechanism and thrombin formation (Fondati et al, 1998). Increased PF-3 levels result in thrombosis, microangiopathic hemolytic anemia, and thrombocytopenia.

Acquired Vascular Wall Disease

Rocky Mountain Spotted Fever • Commonly reported acquired vascular wall diseases, include Rocky Mountain spotted fever, heartworm disease, infectious canine hepatitis, leishmaniasis, neoplasia, purpura hemorrhagica, and various endocrinopathies. Rocky Mountain spotted fever (i.e., *Rickettsia rickettsii*) results in vascular disease approximately 2 weeks after infection. The organism replicates in and alters endothelial cells, which induces platelet and fibrinolytic activation, causing thrombocytopenia and DIC. Hematologic abnormalities include altered vascular permeability, petechiae, ecchymosis, edema, splenomegaly, hyperfibrinogenemia, activation of the fibrinolytic system, thrombosis, and possible DIC (Davidson et al, 1990). Decreased plasma factor VIII and increased factor V activity indicate a poor patient prognosis (Schmaier et al, 2001). Many infectious diseases kill animals by DIC-type episodes secondary to vascular damage, tissue necrosis, or both. A common gross lesion seen at necropsy with septicemia is widespread petechial hemorrhages as the result of DIC.

Heartworm Disease • Adult heartworms reside in the right ventricle and pulmonary artery and can cause partial blood flow obstruction, interference with tricuspid valve closure, with resultant caval syndrome because of retrograde migration of adult heartworms. Adult worms also cause direct endothelial damage, leading to myointimal proliferation, exposure of subendothelial collagen, and hypercoagulability, thrombocytopenia, and possible or pulmonary thromboembolism or DIC. Administration of thiacetarsemide adulticide may contribute to the incidence of thromboembolism via platelet aggregation (Boudreaux and Dillon, 1991).

Leishmaniasis • Hemostatic abnormalities caused by leishmaniasis include epistaxis and thrombocytopenia. DIC may be triggered by endothelial damage. Thrombocytopenia, thrombopathia, and altered plasma fibrin and FDP concentrations have been reported. Epistaxis may be caused by local nasal inflammation in the absence of detectable hemostatic defects.

Acquired Hemostatic Disease • Acquired bleeding disorders are better investigated in people, and these mechanisms should be considered in confusing cases. A relatively rare acquired form of vWD is associated with hematoproliferative disorders, including monoclonal gammopathies, lymphoproliferative disorders, and myeloproliferative disorders in humans. Autoantibodies directed against vWF may lead to its more rapid clearance from the circulation or interference with its function. Other diseases causing acquired hemostatic disorders include neoplasia, which causes altered angiogenesis, alterations in tumor-associated vascular endothelium, hypercoagulability, and venous thromboembolism, partially the result of increased synthesis of thrombin, tissue factor, clotting factors, and vascular endothelial growth factor by tumor cells or tumor-associated cells. Diabetes mellitus and hypoadrenocorticism may alter coagulation, including impaired fibrinolysis, platelet aggregation, and increased plasminogen activator inhibitor. Acquired vascular lesions occur in feline infectious peritonitis (FIP)-associated vasculitis, immune-mediated disease such as hemorrhagic vasculitis (Nazarova et al, 2001), drug reactions, trauma, gastrointestinal ulceration, and vitamin C deficiency.

Disorders of Primary Hemostasis

Thrombocytopenia, an absolute decrease in the number of platelets, is the most common acquired hemostatic disorder of dogs (Grindem et al, 1991). The causes are many and may include decreased platelet production because of infectious, toxic, or neoplastic bone marrow disease or accelerated platelet destruction or use because of immune-mediated or infectious and inflammatory disease. Platelet sequestration in the spleen can be accentuated with splenomegaly, hypothermia, splenic congestion, or splenic hyperplasia. The most common causes of canine thrombocytopenia are immune-mediated, infectious disease and neoplasia, whereas thrombocytopenia in cats is most often the result of infectious disease, neoplasia, or thromboembolism (Hisasue et al, 2001).

NOTE: Thrombocytopenia is the most common cause of clinical signs of bleeding in dogs. Thus platelet numbers should be evaluated first in unexplained bleeding disorders. A platelet estimate from a blood smear is simple, quick, inexpensive, and sufficient to detect severe thrombocytopenia.

Thrombocytopenia is a platelet count less than the appropriate reference range (see Appendix II). One should first confirm that thrombocytopenia is not an error of collection, sample-handling delay, or laboratory error (Figure 5-9). A common cause of pseudothrombocytopenia is platelet clumping, especially in feline samples. One should check blood smears for platelet clumping (see Chapter 2) and collect a fresh sample and repeat the count if in question.

The severity of the thrombocytopenia aids in interpretation and in treatment decisions. Persistent mild thrombocytopenia of 100,000 to 175,000 platelets/µl may not be specific for a particular disease. Platelet counts under 20,000/µl in dogs suggest immune-mediated thrombocytopenia. Clinical signs (e.g., epistaxis, hematochezia, melena, hematuria, hematemesis, cutaneous petechial or ecchymotic hemorrhage) become likely with severe thrombocytopenia (< 20,000 platelets/µl). Some animals may not bleed with 10,000 platelets/µl, whereas others may bleed at 30,000 to 50,000 platelets/µl. Platelet size,

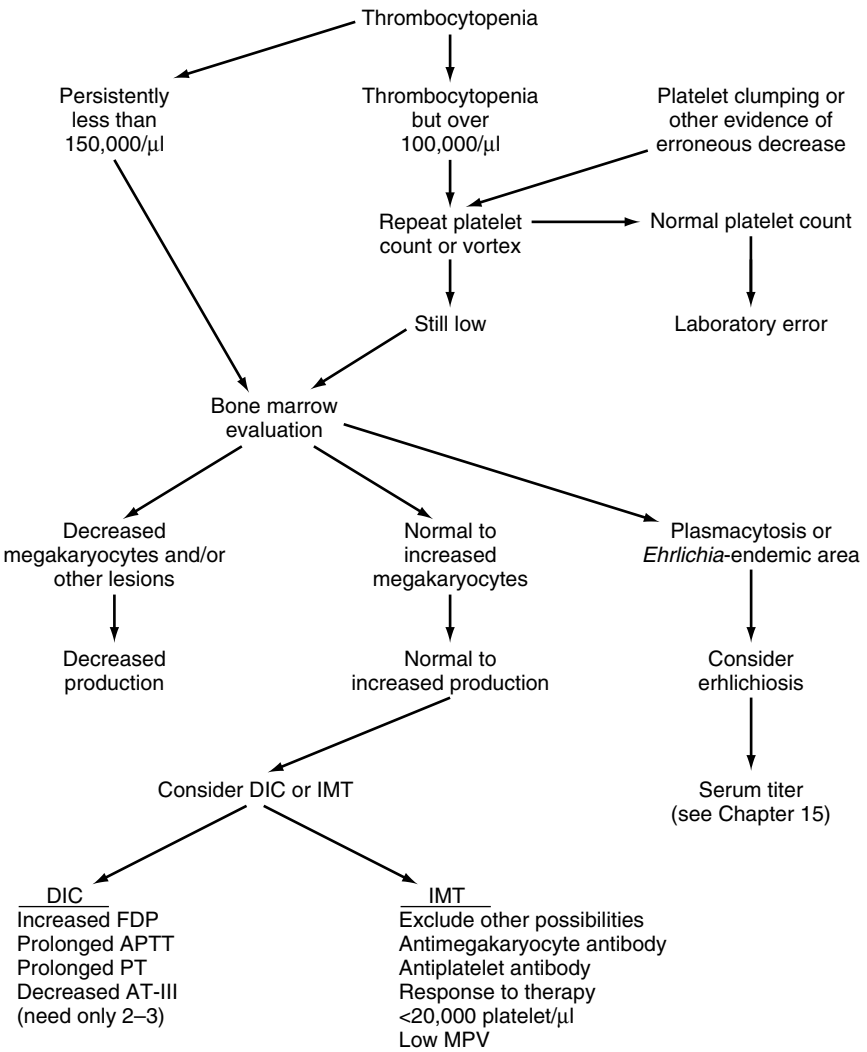

FIGURE 5-9. One diagnostic approach to canine thrombocytopenia. This presumes that disseminated intravascular coagulation (DIC), immune-mediated thrombocytopenia (ITP), bone marrow disease, or ehrlichiosis are the usual causes. Rare causes are not considered. One should first establish that no significant thrombocytopenia as the result of technical or sample error exists. Repeating a platelet count may include trying a different anticoagulant, more careful venipuncture, and more rapid processing of the sample after collection. Vortexing feline blood if platelets clump may reveal the low count was only the result of clumping. Bone marrow evaluation should include an aspirate, histologic biopsy, *and* concurrent complete blood count (CBC) evaluation. A diagnosis of a bone marrow problem is confirmed by decreased megakaryocytes or finding of other disease processes, such as leukemia or myelofibrosis. Ehrlichiosis is suspected if plasmacytosis is in the marrow or if the dog was in an endemic area. Finding normal bone marrow and normal to increased megakaryocytes suggests increased removal of platelets. DIC is diagnosed by alteration in hemostatic profile (see text). ITP is diagnosed most directly by antiplatelet or antimegakaryocytic antibody testing and by supportive evidence discussed in text. If other evidence points to either ITP (e.g., platelet count < 20,000/µl or low MPV) or DIC, which is common in severe inflammatory or malignant neoplastic disease, one should use specific testing and bypass bone marrow evaluation.

platelet function, blood vessel and endothelial support, concentration of FDP and coagulation factors, and severity of the challenge to the hemostatic mechanism all contribute to the presence or absence of bleeding. Linking the severity of change in laboratory data to clinical signs is important. For example, if the platelet count is greater than 50,000/µl and the patient is bleeding, additional factors (e.g., defective platelet function associated with DIC) are likely. In contrast, disorders of coagulation tend to have large areas of hemorrhage, hematomas, body cavity hemorrhages, and hemarthroses rather than petechial and ecchymotic hemorrhages.

The three common causes of thrombocytopenia are (1) destruction (e.g., ITP), (2) bone marrow production defects (e.g., estrogen toxicity, immune-mediated destruction of megakaryocytes, neoplasia), and (3) consumption of platelets (e.g., DIC). *Ehrlichia* infections, Rocky Mountain spotted fever, and other bacterial and viral infections commonly cause thrombocytopenia. These infections should be added to the three common causes in areas where the infections are endemic. Bone marrow aspirate, biopsy, or both usually document platelet production via the estimation of megakaryocytic numbers. Bleeding at the aspiration or biopsy site is usually easily controlled, so bone marrow collection should not be feared (see Figure 5-9). DIC is diagnosed with a profile of hemostatic tests. IMT diagnosis lacks commonly available direct testing.

Thrombopathy is more commonly acquired than inherited, more common in dogs than cats, and characterized by defects in platelet function without alterations in platelet numbers. Thrombopathias can be caused by endocrinopathies, such as diabetes mellitus or hyperadrenocorticism, immune-mediated hemolytic anemia (IMHA), pancreatitis, nephrotic syndrome, and can be associated with thrombosis. Inherited defects may include quantitative or qualitative defects in membrane proteins needed for platelet adhesion, cyclic adenosine monophosphate (cAMP) metabolism, prostaglandin metabolism, granule pools, vWF, and fibrinogen concentrations.

Thrombocytosis is not uncommon and was seen in 5% of canine and 3% of feline hematology samples. Causes include neoplasia, endocrine disease, splenic contraction, splenectomy, and immunosuppressive therapy (e.g., prednisolone, vincristine). Nonspecific bone marrow stimulation occurs during leukocytosis or anemia because of cross-stimulation from cytokines IL-3, IL-6, GM-CSF, and erythropoietin. It also occurs with infectious and inflammatory disease, tumors, chronic blood loss, or endocrinopathies.

Thrombocytosis is typically seen in canine iron deficiency anemia. Thrombocytosis in cats is often associated with myeloproliferative disorders or leukemia. Primary thrombocytosis, otherwise known as *platelet leukemia* or *essential thrombocythemia*, may cause pulmonary, mesenteric, central nervous system (CNS), or portal vein thrombosis, resulting in congestive heart failure, pulmonary hypertension, abdominal pain, vomiting, diarrhea, ascites, or seizures and paresis, depending on the site of thrombosis.

Inherited Disorders of Primary Hemostasis

Several canine breeds have asymptomatic lower circulating platelet counts, including greyhounds (Sullivan, Evans, and McDonald, 1994) and Cavalier King Charles spaniels (Pedersen et al, 2002). The macrothrombocytopenia in the Cavalier King Charles spaniels is an autosomal recessive trait, with a predominance of large platelets in circulation.

Defective platelet function may be attributed to certain drugs or diseases such as vWD (Table 5-5). Platelet dysfunction (i.e., thrombopathia) is documented by a platelet function test (e.g., BMBT, platelet aggregation) in animals with adequate platelet numbers. American cocker spaniels may suffer from severe bleeding after minor trauma, venipuncture, or surgery. Although platelet counts, coagulation profiles, and vWF were normal, bleeding times were prolonged and platelet aggregation in response to ADP and collagen were consistently abnormal, suggesting abnormal platelet function. Further studies demonstrated an abnormal concentration of ADP in dense granules in the platelets, leading to the bleeding diathesis (Callan et al, 1995). Rare disorders of membrane glycoproteins, platelet adhesion, dense body and alpha granule secretion, ATP production, and generation of procoagulant activity include basset hound thrombopathia, Spitz thrombopathia, thrombasthenic thrombopathia of otter hounds, Bernard-Soulier syndrome, Hermansky-Pudlak syndrome, Wiskott-Aldrich syndrome, Glanzmann's thrombasthenia of Great Pyrenees, and dense granular storage pool disease of American cocker spaniels (Boudreaux and Lipscomb, 2001; Boudreaux and Catalfamo, 2001; Nurden and Nurden, 2001).

TABLE 5-5. Selected Causes of Abnormal Platelet Function

CAUSE	
Drugs	Aspirin, ibuprofen, phenylbutazone, indomethacin
Acquired	Lymphoproliferate disorders, disseminated intravascular coagulation (DIC), uremia
Hereditary	vWD and rare disorders in basset hounds, otter hounds, foxhounds, Spitz, Great Pyrenees, Scottish terriers, American cocker spaniels

Acquired Disorders of Primary Hemostasis

Acquired disorders of platelet numbers and function are many and may arise after infectious or neoplastic disease or secondary to hyperthermia or drug therapy. For example, aspirin and nonsteroidal antiinflammatory agents, which irreversibly or reversibly acetylate platelet cyclooxygenase, prevent generation of thromboxane A_2 needed for secretion and aggregation. Bone marrow diseases affecting production may be the result of a variety of toxins, infections, leukemia, immune-mediated damage, as well as other influences.

Toxins include estrogen (including testicular tumors), phenylbutazone, chemotherapeutic agents, and dapsone. Often myelofibrosis or a fatty marrow remains without evidence of the causative agent. A bone marrow aspirate is indicated when a patient exhibits bicytopenia, pancytopenia, or abnormal peripheral blood cell morphology.

Immune-Mediated Thrombocytopenia • ITP is a common disorder in which binding of antibodies against surface glycoproteins responsible for platelet aggregation results in platelet removal and alterations in MPV (Wilkerson et al, 2001). ITP is often diagnosed by ruling out a marrow production problem, DIC, and other common diseases. Increased numbers of activated platelets and response to immunosuppressive therapy (Scott-Moncrieff et al, 2001) further support the diagnosis.

Detection of antiplatelet antibodies on platelets in blood or in serum has been validated in dogs but not in cats. Tests are not widely available but include highly sensitive flow cytometric tests for detection of serum platelet bindable IgG and platelet-bound IgG (Kristensen, Weiss, and Kausner, 1994; Lewis et al, 1995). Antimegakaryocytic antibodies may be identified on bone marrow smears. Two indirect indicators of ITP are (1) a low MPV and (2) a platelet count less than 20,000/µl. ITP may be secondary to drugs, infections, multiple transfusions, or lupus, or it may be idiopathic.

Ehrlichiosis • Thrombocytopenia, anemia, bicytopenia, or pancytopenia in a dog warrant considering *Ehrlichia*. Different species of *Ehrlichia* infect dogs. *Ehrlichia canis* is discussed here. Thrombocytopenia is typically found in ehrlichiosis. The platelet count is often greater than 20,000/µl and yet may still be associated with bleeding (e.g., epistaxis, hyphema, petechia). Platelet dysfunction in canine ehrlichiosis may be caused by antiplatelet antibodies (Harrus et al, 1996). If the animal does not have bone marrow destruction as the result of *Ehrlichia*, the platelet count is usually 75,000 to 175,000/µl. Other hematologic findings associated with ehrlichiosis include mild to severe nonregenerative anemia (approximately 90% of cases), as well as leukopenia and neutropenia (approximately 50% of cases). Neutropenia may be severe enough to allow overwhelming sepsis. In some dogs, the bone marrow has ineffective hematopoiesis with a paradoxic pattern of normal to increased cellularity despite pancytopenia. Anemia is not common in acute cases. Severe anemia and severe leukopenia are generally *only* seen when chronic disease and loss of bone marrow cellularity are found. The acute phase of the disease resembles other infections, with fever, anorexia, weight loss, and lymphadenopathy.

The chronic form is more variable, although hyperproteinemia and hypergammaglobulinemia are frequently present; the latter is occasionally severe enough to induce serum hyperviscosity. Polyclonal gammopathy is expected, but occasional serum protein electrophoretograms with oligoclonal peaks may mimic the monoclonal peaks of a lymphoid neoplasm. Numerous plasma cells in the bone marrow may also mislead one into diagnosing a plasma cell myeloma. The exuberant immune response may lead to an immune-complex glomerular disease and proteinuria.

Hemolytic Uremic Syndrome • Hemolytic uremic syndrome has been reported in humans and animals (i.e., dogs, cats), is associated with acute renal failure and thrombocytopenia after infectious and inflammatory disease, and has been observed after renal transplantation in cats and gastroenteritis in dogs (Holloway et al, 1993; Aronson and Gregory, 1999). Platelet hyperaggregability and platelet thrombi, tissue ischemia, thrombocytopenia, and microangiopathic hemolytic anemia characterize this syndrome. Alteration of the thrombogenic properties of the endothelium with increased expression of tissue factor and enhanced platelet deposition can occur after 24 to 72 hours of exposure to uremic serum.

Retroviral Disease • Cats with feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), or both often have moderate nonregenerative anemia with or without

concurrent granulocytopenia and thrombocytopenia (Shelton and Linenberger, 1995). One should always test for FeLV, FIV, FIP, and rickettsial disease in thrombocytopenic cats. Platelets from FeLV-infected cats or those with DIC may have decreased granularity or vacuolation.

Hyperthermia • Hyperthermia has been used with some success in the treatment of certain types of cancer and viral infections. However, hyperthermia may cause thrombocytopenia, increased plasma FDPs, prolonged clotting times, and evidence of spontaneous bleeding, caused (in part) by hyperthermia-induced liver injury (Diehl et al, 2000).

Drug-Associated Platelet Dysfunction • Drugs that may affect platelet function include corticosteroids, sulfinpyrazone, methylxanthines, furosemide, carbenicillin and other synthetic penicillins, cephalosporins, nitrofurantoin, chloroquine, levamisole, phenothiazine, sympathetic blocking and stimulatory agents, prostacyclin, estrogen, ethanol, caffeine, heparin, antihistamine, dextran, vitamin E, and dipyridamole. A return of normal platelet function 4 to 5 days after discontinuing use of a drug suggests a cause-and-effect relationship. More rigorous evidence is needed to prove the effect.

Disorders of Secondary Hemostasis

Abnormal coagulation test results indicate a coagulopathy (i.e., impaired ability to form a clot). Shortened PT or APTT results are usually ignored, because they do not correlate with clinical hypercoagulability. Abnormal test results (reported in seconds) usually are prolonged (the PT may be reported as a percent of normal from human and European veterinary laboratories). If no reference values are available, the results can be compared with those of a sample from a normal animal run in parallel. A 3-second increase in the PT or a 5-second increase in the APTT over the normal sample usually is significant. Lesser increases are possibly abnormal, but this conclusion must be strengthened with other information or repeated testing.

A defect in the early stage intrinsic pathway (i.e., XII, XI, IX, VIII) would be identified by the combination of a normal PT and prolonged APTT (see Figure 5-8). A defect in factor VII is identified by the combination of a normal APTT and prolonged PT (Figure 5-10).

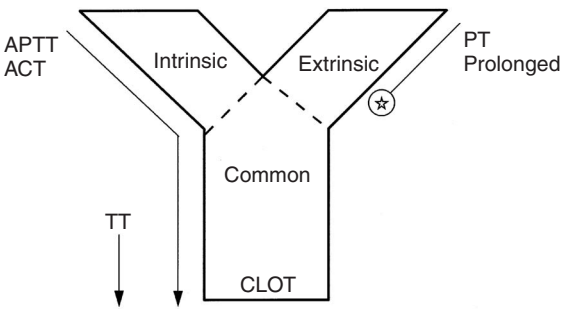

FIGURE 5-10. Test results of a factor VII deficiency. Only the prothrombin time *(PT)* would be abnormal, as depicted by a *star* and *termination of the arrow* at the site of factor VII in the extrinsic system.

A defect in the common pathway (i.e., X, V, prothrombin, fibrinogen) or multiple defects involving the intrinsic, common, and extrinsic pathways would prolong both the PT and APTT. All tests except the TT would have prolonged results in vitamin K antagonist toxicity, because the vitamin K factors (i.e., II, VII, IX, X) would be deficient, creating defects in the intrinsic, extrinsic, and common pathways; however, the concentration of fibrinogen should be adequate (Figure 5-11). Because the liver synthesizes most procoagulant and anticoagulant factors, severe hepatic failure causes multiple defects in the coagulation cascade, including decreased synthesis of clotting and inhibitor factors, decreased clearance of activated factors, quantitative and qualitative platelet defects, hyperfibrinolysis, and accelerated intravascular

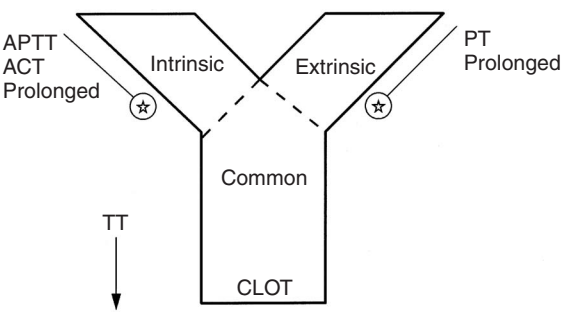

FIGURE 5-11. Test results for vitamin K antagonist toxicity. Inhibition of the vitamin K-related factors (II, VII, IX, and X) in warfarin poisoning creates defects in all three pathways. The thrombin time *(TT)* should be normal, because fibrinogen is not affected. A factor X deficiency would have the same laboratory test pattern, because a defect in the common pathway slows clot formation despite normal intrinsic and extrinsic pathways. Hepatic failure may have a similar pattern unless fibrinogen deficiency is present.

coagulation (Amitrano et al, 2002). DIC is common in animals with hepatic disease. A defect in the common pathway prolongs both the PT and APTT, because the normal intrinsic and extrinsic pathways must pass through the common pathway to form a clot (see Table 5-3).

Clinical signs, breed, sex, and history provide important clues to the diagnosis before specific factor analysis. For example, if only some of the males in a litter of young dogs are affected with severe clinical signs of bleeding (including hematomas) and the dogs have a normal PT but prolonged APTT, the problem is with either factor VIII or factor IX. The combination of prolonged APTT and normal PT has localized the defect to the intrinsic pathway. The historical evidence of a sex-linked hereditary problem has implicated factors VIII and IX, the only two sex-linked defects. The severity of the bleeding rules out factors XII and XI in the intrinsic pathway, because bleeding is absent with factor XII or mild with factor XI deficiency. Factor IX and VIII defects (i.e., hemophilia B and A) have similar clinical bleeding problems and genetic implications. The cost : benefit ratio of more specific testing compared with the economic impact of the diagnosis determines whether additional tests for a more definitive diagnosis are appropriate.

Inherited Disorders of Secondary Hemostasis

Hemophilia A (factor VIII deficiency) is identified by prolongation of the APTT. It is the most commonly reported inherited canine coagulopathy and has been reported cats with multiple factor deficiencies, leading to defects in intrinsic coagulation. The causative mutation has been determined to be an intron 22 inversion found in approximately 45% of severely affected hemophilia A patients (Hough et al, 2002). Several commercial tests have been validated to diagnose factor VIII: C and factor IX deficiencies from the plasma of hemophiliac dogs (Mischke, 2000).

Factor IX deficiency (i.e., hemophilia B or Christmas disease) is sex-linked like hemophilia A and is detected by prolonged APTT. Factor IX deficiency is much less commonly reported and has more consistently severe clinical signs. It affects German shepherds, British shorthairs, Siamese cats, and American domestic shorthaired cats.

Factor X deficiency (i.e., Stuart Prower factor) occurs in Cocker spaniels and Jack Russell terriers (Dodds, 1973). Factor XI deficiency in Kerry blue terriers, Springer spaniels, and Pyrenean mountain dogs, is a severe bleeding disorder with postoperative hemorrhage and prolonged APTT (Knowler et al, 1994). These dogs tend to bleed after trauma or surgery but do not have spontaneous hemorrhage in joints and soft tissue characteristic of hemophiliacs (Gailani, 2001).

Devon Rex cats may have vitamin K-dependent coagulopathies. They are treated successfully with oral vitamin K_1 or plasma transfusion, which normalizes clotting factor II, VII, IX, and X concentrations (Maddison et al, 1990). Prothrombin deficiency occurs in cocker spaniels, boxers and otter hounds and, as a common pathway defect, will have prolonged APTT and PT.

Autosomal dominant factor VII deficiencies have been reported in Alaskan malamutes (Mills, Labuc, Lawley, 1997), Labrador retrievers, and beagles (Spurling, 1988). Factor XII deficiency, or Hageman factor, has been determined to have an autosomal recessive pattern in cats, similar to that which is reported in humans (i.e., Hageman trait) (Kier et al, 1980). A Chinese Sharpei with a combined factor XII deficiency and impaired prekallikrein activity resulted in episodes of intestinal hemorrhage and diarrhea. This is uncommonly reported in the dog, and is not typically associated with hemorrhagic tendencies.

Acquired Disorders of Secondary Hemostasis

Disseminated Intravascular Coagulation • DIC is common, severe, and occurs secondary to a wide variety of diseases. Initiating factors include neoplasia, inflammation, infection, trauma, and certain snake venoms that cause stasis of blood flow, necrotic tissue, rough surfaces, and abnormal vessel linings. The extrinsic system is initiated by tissue thromboplastin from damaged cells; the intrinsic system is activated by abnormal surfaces such as exposed collagen beneath damaged endothelial cells. Inflammatory diseases create areas of necrosis and exposed collagen, which stimulate clotting; many infections, such as canine infectious hepatitis or feline infectious peritonitis (Weiss, Dodds, and Scott, 1980; Wigton, Kociba, and Hoover, 1976), involve a DIC-type episode.

Neoplasms (e.g., hemangiosarcoma) often have necrotic, inflamed areas, and chemotherapy of neoplasms may create additional necrosis and increase the likelihood of DIC. Hemolytic anemias produce abundant

TABLE 5-6. Frequency of Laboratory Abnormalities in Disseminated Intravascular Coagulation

	FREQUENCY OF ABNORMAL RESULTS	
ABNORMALITY	**FELDMAN ET AL, 1981**	**KOCIBA, 1978**
Increased FDP	61%	94%
Prolonged APTT	87%	95%
Prolonged PT	80%	72%
Decreased platelet count	80%	48%

FDP, Fibrin degradation products; *APTT,* activated partial thromboplastin time; *PT,* prothrombin time.
From Feldman BF, Madewell BR, O'Neill MA: Disseminated intravascular coagulation: Antithrombin, plasminogen, and coagulation abnormalities in 41 dogs, *J Am Vet Med Assoc* 179:151, 1981; and Kociba GJ: Disseminated intravascular coagulation proceedings, 29th Annual Meeting, American College of Veterinary Pathologists, San Antonia, TX, 1978.

RBC debris. Substances such as amniotic fluid strongly induce clotting. These explain the high incidence of DIC in hemolytic anemia and obstetric disorders. Vasculitis may induce DIC, because vessels have damaged endothelial cells. Endothelial cells provide a complete barrier between plasma and subendothelial collagen and generate factors that inhibit platelet aggregation. DIC can be localized or chronic and not always disseminated and peracute. Death in many diseases is secondary to infarction, thrombosis, or bleeding because of DIC.

One expects platelets and coagulation factors to be consumed during excessive clotting. Breakdown of these clots increases FDP, which act as anticoagulants and interfere with platelet function and clotting by competing with fibrinogen for platelet membrane receptors. FDP, D-dimer, and FgDP serve as a key diagnostic features. Any or all hemostatic test results may be abnormal, including platelet number, BMBT, PT, APTT, FDP, D-dimer, and FgDP. However, because increased production of coagulation factors and platelets can variably compensate for consumption, no single result is predictable (Table 5-6). Confidence in a DIC diagnosis is increased when both thrombocytopenia and coagulation factor deficiency occur concurrently and as more of the expected abnormalities are detected. Decreased antithrombin III (ATIII) is a sensitive indicator of DIC; hepatic insufficiency and protein-losing states may also decrease ATIII, but ATIII testing is not commonly available.

Vitamin K Antagonism • Warfarin-type anticoagulant rodenticides are common causes of acquired bleeding disorders. Second-generation rodenticides have a long functional half-life (i.e., 15 to 20 days for diphacenone, compared with 40 hours for warfarin). These rodenticides act by causing a functional vitamin K deficiency and reduced hepatic synthesis of functional forms of factors II, VII, IX, and X. Sulfaquinoxaline treatment (i.e., coccidiostat) of dogs may cause bleeding by antagonism of vitamin K. Posthepatic biliary obstruction or severe enteric disease rarely cause vitamin K deficiency. The PT is preferred to monitor the coagulation defect. When factor synthesis is inhibited, the factor with the shortest half-life becomes deficient earliest. The half-life of factor VII is 2 to 4 hours, compared with 14 to 16 hours for factors IX and X and 41 hours for prothrombin (II). Because factor VII is in the extrinsic system, the earliest and greatest relative increase is noted in the PT. The thrombotest or PIVKA (*p*roteins *i*nduced by *v*itamin *K a*bsence or *a*ntagonism) is sensitive for the precursor coagulation proteins from the liver that accumulate and spill into the circulation when vitamin K antagonism exists. After effective vitamin K_1 therapy, the liver can reach maximum prothrombin synthesis in 9 to 11 hours and the PT should be approaching normal within 24 hours.

Other acquired coagulopathies arise secondarily from maldigestive and malabsorptive gastrointestinal disorders, exocrine pancreatic deficiency, infiltrative enteritis, bile duct obstruction, hepatic disease, or because of increased circulating heparin from mast cells.

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Jennifer S. Thomas

Introduction to Serum Chemistries: Artifacts in Biochemical Determinations

ARTIFACTS IN BIOCHEMICAL DETERMINATIONS

Artifacts (i.e., when the measured concentration or activity of an analyte is falsely increased or decreased) make it difficult to interpret results of laboratory tests accurately. The *real* concentration or activity of an analyte is not often reliably estimated once an artifact has occurred. Artifacts may interfere with the measurement of a single analyte or with multiple analytes. If one analyte on a biochemical profile is artifactually altered, all analytes should be closely evaluated to determine whether they have also been affected.

Artifacts are more likely if a sample has hemolysis, hyperbilirubinemia, or lipemia. For analytes that are very labile at room temperature and prone to *in vitro* loss (e.g., ammonia), a significant delay in processing or improper handling (e.g., not keeping the sample at the proper temperature) often causes the measured concentration to be inaccurate. Another sample should be collected. Artifacts should also be suspected if the laboratory findings do not correlate with the patient's clinical presentation (e.g., clinically normal patient with markedly increased potassium or decreased glucose concentrations). Finally, artifacts are expected in patients who are receiving a drug known to interfere with the determination of a chemical parameter. For example, chloride concentration usually cannot be accurately measured in patients receiving potassium bromide because the most commonly available techniques cannot distinguish bromide from chloride.

When an artifact is suspected, it is important to determine whether the artifact resulted from a preanalytical or analytic problem (MacWilliams and Thomas, 1992) because the type of problem will determine how to correct it. Preanalytical problems occur with blood collection or handling before the laboratory conducts the actual chemical analysis of the sample (Table 1). Incorrect labeling of samples can cause biochemical values to be reported for the wrong patient. Collection of blood from a catheter can cause dilution of blood if an appropriate volume of catheter fluid is not first discarded. Collection of blood into the wrong anticoagulant (Table 2) interferes with some biochemical assays (Henry and Kurec, 1996). Delayed separation of plasma or serum from blood cells causes (1) leakage of intracellular constituents (e.g., magnesium, potassium, phosphorus, aspartate aminotransferase [AST], lactic dehydrogenase [LDH]) that may falsely increase serum or plasma concentrations; and (2) consumption of glucose that will falsely decrease measured values (MacWilliams and Thomas, 1992). Exposure to excessive heat or cold can cause leakage of intracellular constituents or alter the concentration or activity of temperature-sensitive analytes. Improper patient preparation includes feeding before blood collection or improper timing of blood collection (e.g., post-prandial bile acid determination). At times, a patient's sample may contain endogenous (e.g., hemolysis) or exogenous (e.g., drug) substances that interfere with biochemical analyses. Analytic problems occur during actual performance of a laboratory assay. Operator errors include improper sample aliquoting, incorrect analyzer use, or improper reagent handling. Reagent problems include using out-of-date or improperly stored reagents. Analyzer malfunctions may affect

TABLE 1. Common Causes for Artifacts in Clinical Chemistry Testing

Preanalytical Problems
Incorrect sample labeling
Improper collection via catheter
Wrong anticoagulant
Delayed removal of serum or plasma from cells
Improper sample handling or storage
Inadequate patient preparation
Interfering substances in sample
Analytical Problems
Operator errors
Reagent problems
Analyzer malfunction

the measurement of a single analyte or multiple analytes.

If an artifact is suspected, the first step is to repeat the analysis using the same sample. If the suspect results do not repeat, the usual cause is operator error. If the suspect results repeat on the second analysis, a new blood sample should be collected and the analysis should be repeated. If the suspect results do not repeat on the new sample, then a preanalytical error is likely. If the suspect results repeat, then there is either an analytic problem or the results accurately reflect the patient's status. In the latter situation, the reagent and analyzer function should be evaluated, and the sample should be sent to a referral laboratory for analysis.

TABLE 2. Anticoagulant Effects on Clinical Chemistry Testing

Preferred samples for chemistry testing
Serum: red top tube
Heparinized plasma or whole blood: green top tube
EDTA (sodium or potassium): purple top tube
Binds divalent cations: decreases calcium and magnesium concentrations
Interferes with enzyme activities: is methodology dependent
Increased sodium or potassium concentration
Sodium citrate: light blue top tube
Binds divalent cations: decreases calcium and magnesium concentrations
Interferes with enzyme activities: is methodology dependent
Oxalate (sodium or potassium) or fluoride (sodium): gray top tube
Is used for glucose or lactate determinations only
Increases sodium or potassium concentration
Decreases calcium concentration
Shifts water from cells into plasma: causes dilution effects
Inhibits enzyme activities (e.g., alkaline phosphatase, amylase, lactic dehydrogenase)

EDTA, Ethylenediaminetetraacetic acid.

Hemolysis

Hemolysis is common in blood samples and is recognized by reddish discoloration of plasma or serum. It can cause significant artifacts in a biochemical panel, the severity of which depends on the species involved and the analytical method used. Hemolysis occasionally occurs *in vivo*; however, it more commonly occurs as a result of *in vitro* erythrocyte damage associated with improper sample handling or collection. Hemolyzed samples are best discarded. *In vitro* hemolysis can be minimized with the following: (1) use of sharp needles to collect blood; (2) avoidance of excess negative pressure during blood collection; (3) gentle mixing and handling of tubes after collection; (4) proper centrifugation techniques to separate plasma or serum from cells; (5) prompt removal of serum or plasma from cells; (6) prevention of overheating or freezing; and (7) avoidance of moisture in the specimen.

Hemolysis may falsely increase the measured serum concentration of substances that are present in higher concentrations in the erythrocyte cytoplasm than in plasma (O'Neill and Feldman, 1989; Alleman, 1990). These changes vary according to species or breed and are independent of the analyzer or methodology used (Table 3). Hemolysis also releases free hemoglobin that may alter spectrophotometric assays measuring wavelengths within the absorbance range of hemoglobin. Finally, hemolysis releases erythrocyte contents that may directly interact with an analyte or interfere with chemical reactions used to determine analyte concentration. The artifacts associated with these last two hemolysis effects vary significantly, depending on the specific analyzer and methodology used (Figure 1). Therefore referral laboratories or analyzer manufacturers should provide information about the effects of hemolysis on their specific analytic system.

Other Causes of Color Interference

Similar problems may occur as a result of color interference associated with hyperbilirubinemia or with the administration of bovine

TABLE 3. Hemolysis Effects on Clinical Chemistry Testing

Analytes Altered as a Result of Release from Erythrocytes: Species and Breed Dependent
AST
CK
Lactic dehydrogenase
Phosphorus
Potassium*
Analytes Altered as a Result of Interference with Assay: Analyzer or Methodology Dependent
Alanine aminotransferase
Albumin
Alkaline phosphatase
Amylase
AST
Calcium
Chloride
Cholesterol
CK
Creatinine
Glucose
Lipase
Total bilirubin
Total protein

*Altered only in those breeds with high erythrocyte concentrations of potassium. Should not be altered in most dogs and cats.
AST, Aspartate aminotransferase; *CK*, creatine kinase.

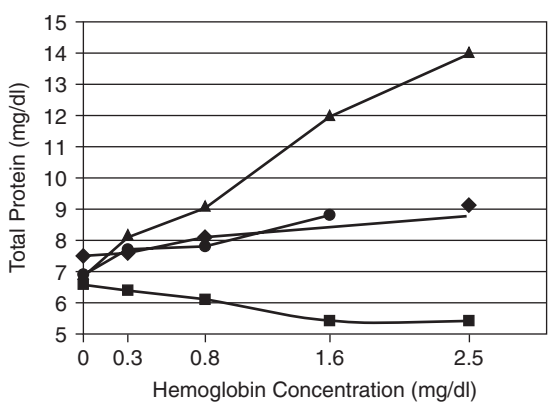

FIGURE 1. The effect of adding varying amounts of hemolysate (as indicated by the concentration of hemoglobin) to normal canine serum on the measured concentration of total protein using 4 different chemistry analyzers. ▲ Model 8000 Bichromatic Analyzer (Boehringer Mannheim Diagnostics, Inc., Indianapolis, IN); ● SMA-2C (Technicon Instruments Corp., Tarrytown, NY); ■ DACOS (Coulter Electronics, Inc., Hialeah, FL); ◆ SMA 12/60 (Technicon Instruments Corp.). (Figure created using data from O'Neill et al., 1989).

hemoglobin solutions (e.g., Oxyglobin, Biopure Corporation). The addition of bovine hemoglobin solutions to human plasma causes significant alterations of some analytes. The analytes affected vary significantly, according to the analyzer used (Ma et al, 1997). The activity or concentration of some analytes increases whereas others decrease. Similar published studies are not available for veterinary species.

Lipemia

Lipemia causes serum or plasma to appear milky white and turbid when triglyceride concentrations exceed 300 to 400 mg/dl. Lipemic samples should be discarded because lipemia alters the light-scattering property of blood (Alleman, 1990), causing alterations of a variety of chemical analytes, depending on the methodology and analyzer used. Analytes falsely reported to be increased by lipemia include total protein, total bilirubin, albumin, globulin, glucose, calcium, and phosphorus. Those reported to be decreased by lipemia include lipase, alanine aminotransferase (ALT), AST, alkaline phosphatase (ALP), and amylase (Alleman, 1990). In addition, lipemia causes *in vitro* hemolysis that can compound the artifacts.

Recent feeding is the most common cause of lipemia. Fasting 12 hours before blood collection can usually prevent postprandial lipemia. If lipemic samples are unavoidable (e.g., patient with a lipid metabolism disorder), then the serum or plasma can be cleared by ultracentrifugation or by the addition of a polymer that binds lipids for removal by centrifugation (e.g., LipoClear, StatSpin). Ultracentrifugation is the standard by which other lipid-clearing methods are judged and is used by many referral laboratories; however, ultracentrifugation is not available in most practices. When using clearing polymers, it is important to first determine whether the procedure will falsely alter the concentration or activity of measured analytes for the analyzer system used.

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6

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Electrolyte and Acid-Base Disorders

Electrolyte and acid-base disorders may result from many different diseases. Timely correction of fluid, electrolyte, and acid-base disturbances is often of more immediate benefit to patients than a specific diagnosis, although both are desirable.

SERUM POTASSIUM CONCENTRATION

Commonly Indicated • Common indications to measure serum potassium concentration include prolonged anorexia, vomiting, diarrhea, muscle weakness, bradycardia, supraventricular arrhythmias, oliguria, anuria, and polyuria. Serum potassium concentrations should be measured if hypoadrenocorticism, acute or chronic renal failure, diabetic ketoacidosis, prolonged vomiting, urethral obstruction, uroabdomen, or postobstructive diuresis are suspected, or if use of diuretics (e.g., furosemide, thiazides, spironolactone) or angiotensin-converting enzyme inhibitors (e.g., enalapril) has occurred.

Analysis • Serum potassium concentrations are measured in serum, plasma, or urine by dry reagent methods, ion-specific potentiometry, and flame photometry (rarely used now). Different methods provide comparable results. Measured potassium concentrations obtained with the new "point-of-care" instruments do not always correlate well with results determined by traditional analyzers. Point-of-care units that measure potassium in whole blood typically give results approximately 0.5 mEq/L less than that obtained with other instruments.

Normal Values • Dogs and cats, 3.5 to 5.5 mEq/L (mEq/L are the same as mmol/L for univalent ions).

Danger Values • Concentrations less than 2.5 mEq/L (muscle weakness) or greater than 7.5 mEq/L (cardiac conduction disturbances) are considered dangerous. Severely hyponatremic animals seem less able to compensate for hyperkalemia.

Artifacts • Serum potassium concentrations exceed plasma concentrations because potassium is released from platelets during clotting. This difference is most pronounced when thrombocytosis occurs. Hemolysis causes hyperkalemia *if* red blood cells (RBCs) have a high potassium content. Most dog and cat RBCs contain little potassium; however, some (e.g., neonates, Akitas, English springer spaniels) have a higher potassium content (i.e., ≥ 20 mEq/L), and hemolysis may cause hyperkalemia. Animals with white blood cell (WBC) counts greater than 100,000/µl may have enough WBCs lyse and release potassium during clotting that serum potassium is artifactually raised. These are causes of *pseudohyperkalemia,* because they only occur *in vitro.* Using lithium heparin tubes for collection plus prompt

separation of plasma from cells prevents these problems. Samples contaminated by drawing them through improperly cleared intravenous (IV) catheters may yield falsely increased or decreased potassium concentrations, depending on the fluid being administered. When obtaining blood from an IV catheter, one should remove and discard enough blood to clear the catheter before collecting the sample. Using ethylenediaminetetraacetic acid (EDTA) or potassium oxalate as an anticoagulant may markedly alter measured values. Large amounts of bilirubin may slightly increase potassium concentrations measured with ion-selective electrodes.

Drugs That May Alter Serum Potassium Concentration • Hypokalemia may be caused by administration of furosemide, thiazides, acetazolamide, laxatives, mineralocorticoids (e.g., fludrocortisone, desoxycorticosterone pivalate), insulin, sodium bicarbonate, amphotericin B, large doses of sodium penicillin G given IV, chronic administration of ammonium chloride, potassium-free fluids, and glucose-containing crystalloid solutions. Peritoneal dialysis can be responsible if potassium-free dialysate is used long-term.

Hyperkalemia may be caused by excessive potassium chloride (either IV or oral), heparin solutions containing chlorbutol, massive digitalis overdose, and potassium penicillin G given IV. It may also be caused by trimethoprim, angiotensin-converting enzyme inhibitors (e.g., enalapril), blood transfusions (if from a dog with high intracellular potassium), potassium-sparing diuretics (e.g., spironolactone, amiloride), mannitol infusions causing acute hypertonicity, nonspecific beta blockers, and nonsteroidal antiinflammatory drugs (if they cause renal failure).

Causes of Hypokalemia • The three possible mechanisms for hypokalemia are (1) decreased intake, (2) translocation of potassium from extracellular to intracellular fluid, and (3) loss via the kidneys or gastrointestinal tract (Box 6-1 and Figure 6-1). Dilution of serum potassium concentration by giving potassium-free fluids, especially those containing glucose, may contribute to hypokalemia. Decreased intake may aggravate hypokalemia as the result of increased loss or translocation but is unlikely to cause hypokalemia by itself. Hypokalemia often results from a combination of decreased intake plus urinary or gastrointestinal losses (e.g., administering potassium-free fluids to anorexic animals).

Translocation of potassium from extracellular to intracellular fluid may occur with bicarbonate administration or insulin-mediated glucose uptake by cells. Both situations typically are iatrogenic (e.g., aggressive treatment

BOX 6-1. Causes of Hypokalemia

Pseudohypokalemia *(infrequent and rarely causing significant change)*

Increased Loss (most common and important category)

Gastrointestinal ($FE_K < 6\%$)
Vomiting of gastric contents *(common and important)*
Diarrhea *(common and important)*

Urinary (FEk > 20%)

Chronic renal failure in cats *(common and important)*
Diet-induced hypokalemic nephropathy in cats *(important)*
Postobstructive diuresis *(common and important)*
Inappropriate fluid therapy (especially with inadequate potassium supplementation) *(common and important)*
Diuresis caused by diabetes mellitus/ketoacidosis *(common and important)*
Dialysis *(uncommon)*
Drugs
  Loop diuretics (e.g., furosemide) *(common and important)*
  Thiazide diuretics (e.g., chlorothiazide, hydrochlorothiazide)
  Amphotericin B
  Penicillins *(rare)*
  Albuterol overdose *(rare)*
Distal (type I) RTA *(rare)*
Proximal (type II) RTA after $NaHCO_3$ treatment *(rare)*
Mineralocorticoid excess *(rare)*
Hyperadrenocorticism (mild changes)
  Primary hyperaldosteronism (i.e., adenoma, hyperplasia)

Translocation *(extracellular fluid → intracellular fluid)*

Glucose-containing fluids ± insulin *(common and important)*
Total parenteral nutrition solutions *(uncommon, but important)*
Alkalemia *(uncommon)*
Catecholamines *(rare)*
Hypokalemic periodic paralysis (Burmese cats) *(rare)*
Hypothermia *(questionable)*

Decreased Intake

Unlikely to cause hypokalemia by itself unless diet is severely deficient
Administration of potassium-free fluids (e.g., 0.9% NaCl, 5% dextrose in water)

FE_k, Fractional excretion of potassium; *RTA*, renal tubular acidosis.
Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 93.

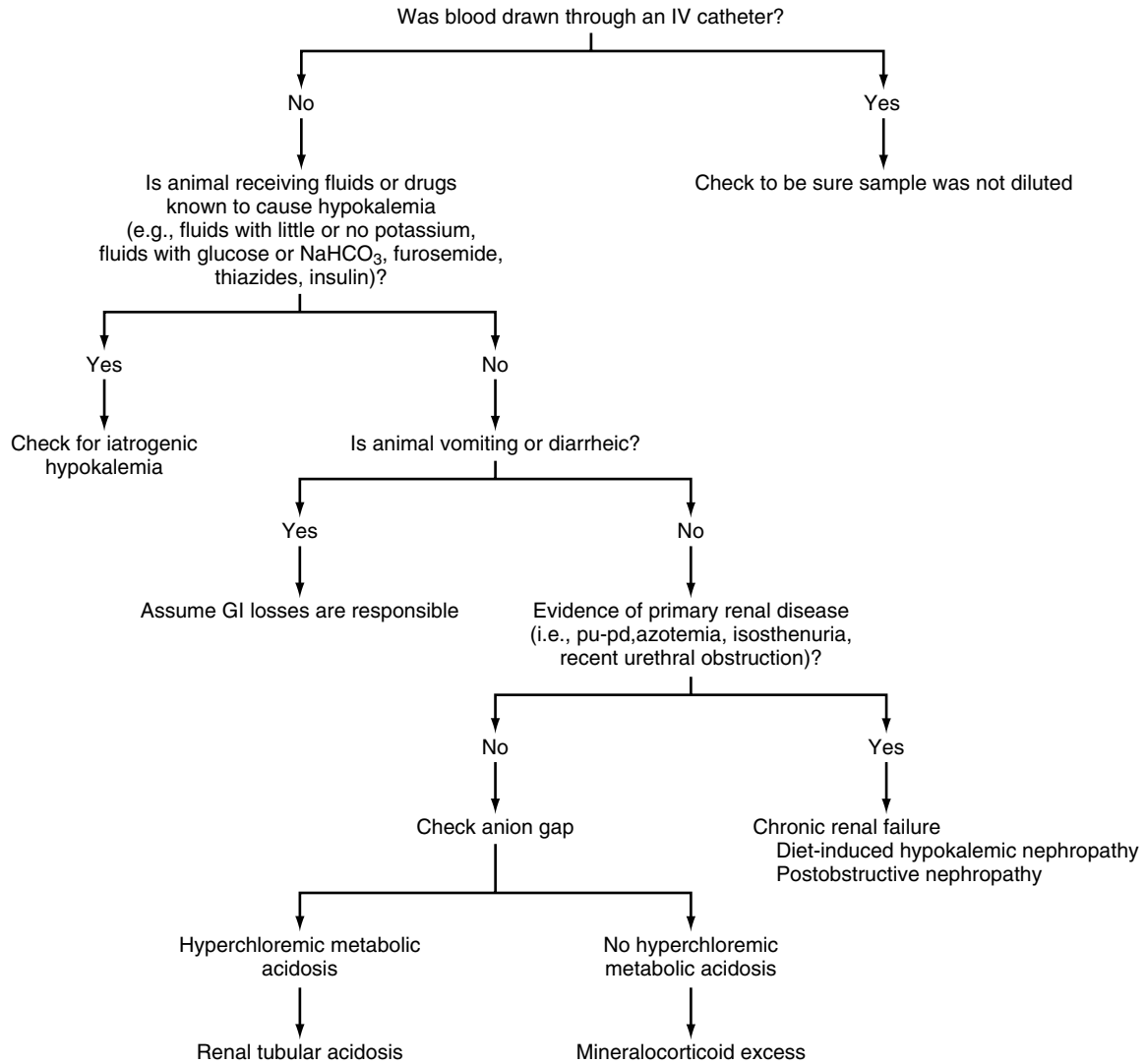

FIGURE 6-1. Algorithm for clinical approach to hypokalemia. *GI,* Gastrointestinal; *IV,* intravenous; *pu-pd,* polyuria-polydipsia. (Modified from DiBartola SP: *Fluid therapy in small animal practice,* Philadelphia, 1992, WB Saunders, p 98.)

for diabetic ketoacidosis). Total parenteral nutrition may do likewise if sufficient potassium is not present in the solution. Hypothermia may cause potassium to enter cells (this effect is reversed when hypothermia is corrected). Hypokalemic periodic paralysis in young Burmese cats causes potassium to move intracellularly and is characterized by recurrent episodes of limb muscle weakness and neck ventriflexion, increased creatine kinase concentrations, and hypokalemia.

Excessive gastrointestinal (e.g., vomiting, diarrhea) and urinary (e.g., polyuria) losses commonly cause hypokalemia. Vomiting gastric contents causes loss of potassium and chloride. The resulting hypochloremia and metabolic alkalosis causes additional urinary loss of potassium and hydrogen. Aldosterone secretion because of dehydration from any cause results in sodium retention but further potassium excretion. Loop diuretics (e.g., furosemide) cause renal potassium wasting. Hypokalemia occurs in approximately 20% to 30% of cats and 10% of dogs with chronic renal failure.

Hypokalemic nephropathy characterized by tubulointerstitial nephritis may develop in

cats fed high-protein diets with inadequate potassium, especially with diets that also contain urinary acidifiers.

Hypokalemia commonly occurs during the postobstructive diuresis after relief of feline urethral obstruction. Hypokalemia may occur in canine hyperadrenocorticism because of mineralocorticoid effects of endogenous steroids and is more common with adrenal tumors than in pituitary-dependent disease.

The most common causes of moderate-to-severe hypokalemia (i.e., < 2.5 to 3.0 mEq/L) are vomiting of gastric contents, urinary losses (e.g., postobstructive diuresis, polyuric chronic renal failure), use of loop diuretics (especially in anorexic animals), aggressive insulin and sodium bicarbonate therapy (e.g., treatment of diabetic ketoacidosis), and inappropriate fluid therapy in anorexic animals. Causes of hypokalemia can usually be ascertained from history and physical examination. Additional laboratory tests are rarely needed.

Causes of Hyperkalemia • The three mechanisms for hyperkalemia are (1) increased potassium intake, (2) translocation of potassium from intracellular to extracellular fluid, and (3) decreased urinary potassium excretion (most common) (Box 6-2 and Figure 6-2). Increased intake is seldom the cause, unless potassium administration is greatly excessive or concurrent renal or adrenal impairment exists.

Translocation of potassium from cells to extracellular fluid may occur with acute inorganic acidosis, massive tissue damage (e.g., acute tumor lysis) or potassium retention (caused by acute renal failure), insulin deficiency, and acute hypertonicity. Acute acidosis as the result of inorganic acids (e.g., NH_4Cl, HCl) but not organic acids (e.g., lactic acid, keto acids) may cause potassium to shift out of cells (uncommon). The effect of inorganic metabolic acidosis on serum potassium concentration varies, usually raising potassium 0.17 to 1.67 (mean, 0.75) mEq/L per 0.1 unit decrement in pH; however, this rule of thumb is not reliable. Respiratory acidosis has minimal effect on potassium. Acute tumor lysis syndrome rarely occurs after radiation or chemotherapy for lymphoma. Other causes of massive tissue damage include reperfusion injury and crush injury (rare). Insulin deficiency and hyperosmolality may cause hyperkalemia in diabetic ketoacidosis. Acute hypertonicity (e.g., mannitol infusion, hyperglycemia) may cause water and potassium to exit cells and enter the extracellular space, causing hyperkalemia (uncommon).

Decreased excretion is the most important mechanism; hyperkalemia seldom occurs if renal function is normal. The most common causes of decreased urinary potassium excretion are urethral obstruction, ruptured bladder (or ureter), anuric or oliguric renal failure, and hypoadrenocorticism.

BOX 6-2. Causes of Hyperkalemia

Pseudohyperkalemia
- Thrombocytosis *(usually mild, but can have marked changes)*
- WBCs > 100,000/µl *(rare cause, but can cause significant changes)*
- Hemolysis in breeds or individuals with high RBC potassium concentration (e.g., Akitas, English springer spaniels, neonates, occasional other dogs)

Decreased Urinary Excretion *(most common)*
- Urethral obstruction *(common and important)*
- Ruptured bladder/ureter *(uncommon but important)*
- Anuric or oliguric renal failure *(common and important)*
- Hypoadrenocorticism *(uncommon but important)*
- Selected gastrointestinal diseases (e.g., trichuriasis, salmonellosis, perforated duodenal ulcer)
- Chylothorax with repeated pleural fluid drainage *(rare)*
- Hyporeninemic hypoaldosteronism (with diabetes mellitus or renal failure) *(rare)*
- Drugs (angiotensin-converting enzyme inhibitors [e.g., enalapril],* potassium-sparing diuretics [e.g., spironolactone, amiloride, triamterene],* prostaglandin inhibitors,* heparin*)

Increased Intake
- Unlikely with normal renal/adrenal function, unless administration is greatly excessive (e.g., intravenous [IV] administration of fluids with high KCl concentrations, administration of large doses of potassium penicillin G)

Translocation (intracellular fluid → extracellular fluid)
- Insulin deficiency (e.g., diabetic ketoacidosis) *(uncommon and transient)*
- Acute inorganic acidosis (e.g., HCl, NH_4Cl) *(rare)*
- Massive tissue damage (e.g., acute tumor lysis syndrome *[rare]*, reperfusion of extremities after aortic thromboembolism in cats with cardiomyopathy *[rare]*, crush injuries)
- Hyperkalemic periodic paralysis *(rare)*
- Drugs (nonspecific beta blockers [e.g., propranolol*])

*Only likely to cause hyperkalemia in conjunction with other contributing factors (e.g., decreased renal function, concurrent administration of potassium supplements).
WBC, White blood cell; *RBC*, red blood cell.
Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 100.

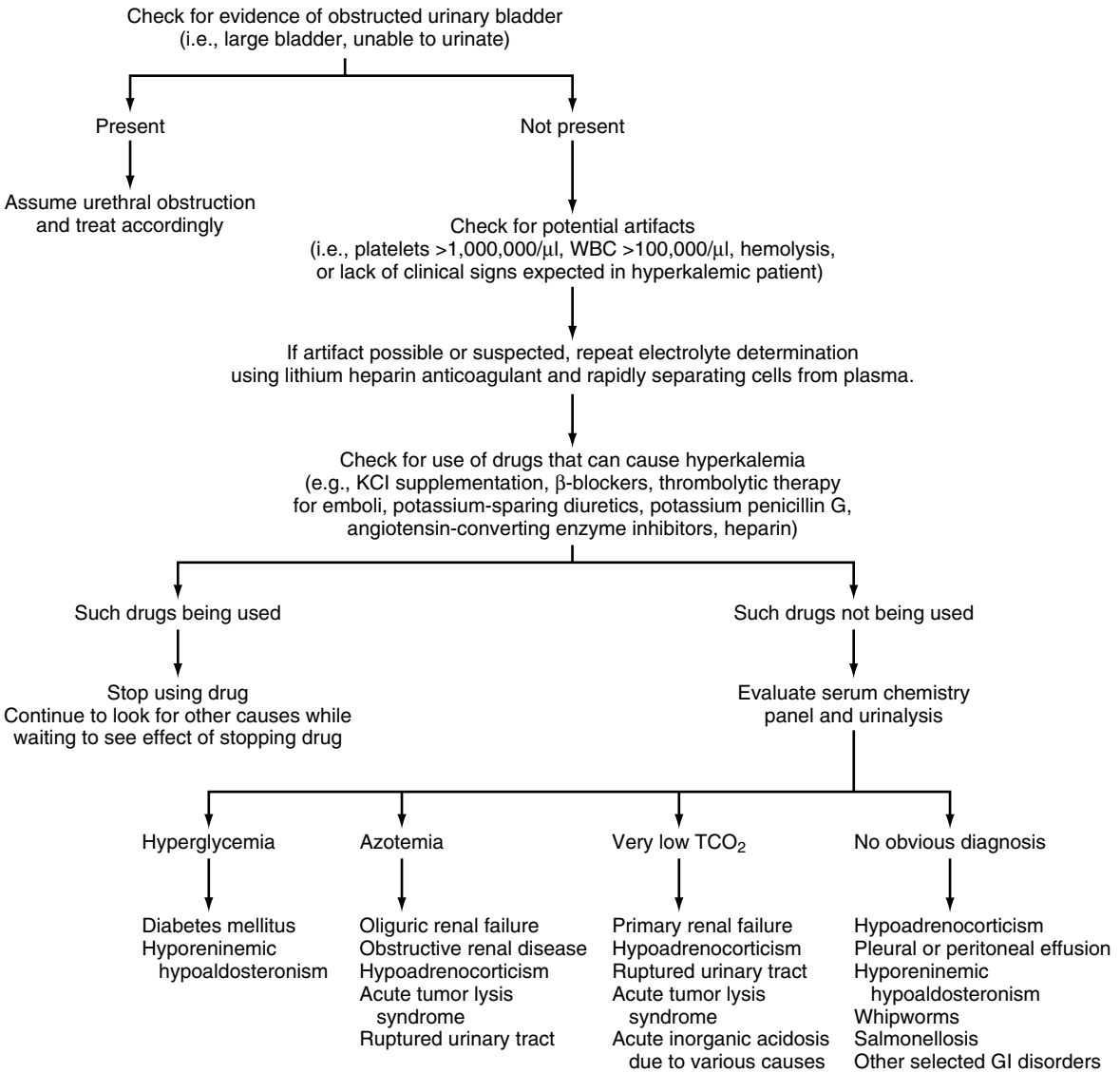

FIGURE 6-2. Algorithm for clinical approach to hyperkalemia. *GI,* Gastrointestinal; *WBC,* white blood cell. (Modified from DiBartola SP: *Fluid therapy in small animal practice,* Philadelphia, 1992, WB Saunders, p 107.)

Hyperkalemia may occur within 48 hours of feline urethral obstruction, but it does not usually occur for at least 48 hours after urinary bladder rupture. Hyperkalemia seldom occurs in chronic renal failure and then usually only in oliguric patients. Hyperkalemia, hyponatremia, and Na/K ratios less than 27 : 1 are often (not always) found in animals with hypoadrenocorticism or renal failure. An adrenocorticotropic hormone (ACTH)-stimulation test (see Chapter 8) is necessary to diagnose hypoadrenocorticism, because identical electrolyte abnormalities can occur because of renal failure, whipworms, salmonellosis, and pleural or peritoneal effusions.

Very rarely, hyporeninemic hypoaldosteronism impairs urinary potassium excretion, causing hyperkalemia in patients with diabetes or renal failure. This disease is diagnosed by measuring aldosterone (not cortisol) concentrations before and after ACTH administration. Hyperkalemic periodic paralysis is another rare cause of hyperkalemia that has been reported in only one dog.

The most important causes of serious hyperkalemia (i.e., > 6.0 mEq/L) are oliguric

and anuric acute renal failure (e.g., ethylene glycol ingestion), urethral obstruction in male cats, and hypoadrenocorticism. Pseudohyperkalemia should be eliminated first. If serum potassium concentration is greater than 7.0 mEq/L and the patient is asymptomatic (e.g., normal electrocardiogram and physical examination), serum potassium concentration should be rechecked using lithium heparin plasma. After artifact has been eliminated, history should be examined for iatrogenic causes. If hyperkalemia might be iatrogenic, the drug in question should be discontinued and serum potassium rechecked in 1 to 2 days. Diagnostic evaluation should continue in case another disease is present, however. Hyperkalemia is usually an indication for evaluation of some or all of the following: serum creatinine, blood urea nitrogen (BUN), urinalysis, and an ACTH-stimulation test.

URINARY FRACTIONAL EXCRETION OF POTASSIUM

Seldom Indicated • Fractional excretion of potassium (FE_K) helps distinguish renal from nonrenal potassium loss. FE_K is calculated as follows:

$$[(U_K/S_K)/(U_{Cr}/S_{Cr})] \times 100$$

where

U_K = urine concentration of potassium (mEq/L)

S_K = serum concentration of potassium (mEq/L)

U_{Cr} = urine concentration of creatinine (mg/dl)

S_{Cr} = serum concentration of creatinine (mg/dl)

Normal Values • Dogs and cats, 6% to 20%.

Abnormalities • FE_K should be less than or equal to 6% if the animal has nonrenal sources of potassium loss (e.g., gastrointestinal loss). Values greater than 20% in hypokalemic patients with normal renal function indicate excessive renal potassium losses.

SERUM SODIUM CONCENTRATION

Commonly Indicated • Serum sodium determination is useful in systemic diseases characterized by vomiting, diarrhea, polydipsia and polyuria, muscle weakness, abnormal behavior, abnormal mentation, seizures, edema, pleural or peritoneal effusion, or dehydration. Serum sodium should be determined whenever adrenal, renal, hepatic, or cardiac failure has been diagnosed; in cases of prolonged fluid or diuretic therapy; or in patients that are not drinking water. Results obtained by using point-of-care instruments usually correlate well with results obtained by traditional instruments.

Analysis • Serum sodium is measured in serum, plasma, or urine by ion-specific potentiometry and dry reagent methods.

Normal Values • Dogs, 140 to 150 mEq/L; cats, 150 to 160 mEq/L (mEq/L are the same as mmol/L).

Danger Values • Clinical signs of hyponatremia and hypernatremia are more related to rapidity of onset than to magnitude of change and associated plasma hypoosmolality or hyperosmolality. Neurologic signs (e.g., disorientation, ataxia, seizures, coma) may occur at serum sodium concentrations less than 120 or greater than 170 mEq/L in dogs.

Artifacts • Historically, when flame photometry or indirect potentiometry was used and hyponatremia plus normal plasma osmolality was found, this was called *pseudohyponatremia* and was caused by hyperlipidemia or severe hyperproteinemia. Excessive lipid and protein in serum cause the machine to inaccurately determine the concentration. Pseudohyponatremia rarely occurs when ion-specific electrodes are used. Hyperviscosity caused by hyperproteinemia can lead to "short samples" (and artifactual hyponatremia) with certain aspiration techniques. Samples drawn through improperly cleared IV catheters may yield falsely increased or decreased sodium concentrations, depending on the fluid being administered. When obtaining blood from an IV catheter, one should remove and discard enough blood to clear the catheter before collecting the sample. Sodium salts of various anticoagulants (e.g., oxalate, fluoride, citrate) increase measured values.

Drugs That May Alter Serum Sodium Concentration • Hyponatremia may develop because of thiazides, furosemide,

spironolactone, or trimethoprim combined with a diuretic. Drug-induced *syndrome of inappropriate antidiuretic hormone secretion (SIADH)* is reported in people (e.g., vincristine), but not dogs or cats. Hypernatremia may develop because of desoxycorticosterone acetate or pivalate, fludrocortisone, sodium bicarbonate, lactulose, inappropriate therapy with physiologic or hypertonic saline solutions, or sodium phosphate enemas.

Abnormal Serum Sodium Concentrations • Serum sodium concentration is the amount of sodium relative to the volume of water in the blood; it does not reflect total body sodium content. Hyponatremic and hypernatremic patients may have decreased, normal, or increased total body sodium. Hypernatremia almost always causes hyperosmolality, whereas hyponatremia usually implies hypoosmolality.

Causes of Hyponatremia • Accurate evaluation of hyponatremia requires measuring plasma osmolality. Most hyponatremic patients are hypoosmolar, but hyperglycemia (i.e., diabetes mellitus) or mannitol administration (Box 6-3) may cause hyponatremia with hyperosmolality. The next step in evaluating hyponatremia is to estimate hydration status. History may indicate fluid loss. Physical examination allows some evaluation of a patient's hydration status (e.g., skin turgor, moistness of mucous membranes, capillary refill time, pulse rate and character, appearance of jugular veins, presence or absence of ascites).

Dehydrated hyponatremic patients have lost water and sodium but more sodium than water. Nonrenal or renal routes may have lost sodium-rich fluid. Nonrenal losses may be gastrointestinal (e.g., vomiting, diarrhea), third space (e.g., pancreatitis, peritonitis, uroabdomen, pleural effusion), or cutaneous (e.g., burns). Gastrointestinal fluid losses may lead to hyponatremia if the loss of sodium is greater than the loss of water or if subsequent replacement of the lost fluids by drinking water dilutes the remaining sodium. Hypoadrenocorticism, diuretics, diabetes mellitus, or renal disease may cause renal fluid and salt loss. Once again, drinking water replaces water but not sodium, causing hyponatremia. Hyponatremia also has been associated with chronic hemorrhage and hemoabdomen in dogs.

Overhydrated hyponatremic patients (e.g., ascites, edema) may have increased total body sodium. Impaired water excretion causes fluid retention, which dilutes serum sodium. Clinical signs of hypervolemia may not be visible, because the retained water may be intracellular or interstitial. Hypervolemic hyponatremia primarily occurs in congestive heart failure, severe hepatic disease, nephrotic syndrome, and advanced renal failure.

Normovolemic hyponatremia may be caused by primary (i.e., psychogenic) polydipsia, fluid therapy (e.g., 5% dextrose or 0.45% saline), SIADH (rare), drugs with antidiuretic effects, and myxedema coma from hypothyroidism (rare). Primary polydipsia (see Chapter 7) usually occurs in large breeds of dogs. These dogs have severe polydipsia, polyuria, severe

BOX 6-3. Causes of Hyponatremia

With Normal Plasma Osmolality (Pseudohyponatremia) *(rare with current instruments)*
Hyperlipidemia
Marked hyperproteinemia *(rare)*

With High Plasma Osmolality
Hyperglycemia *(common)*
Mannitol infusion

With Low Plasma Osmolality
Overhydration (i.e., hypervolemia)
Severe liver disease causing ascites *(common)*
Congestive heart failure causing effusion *(common)*
Nephrotic syndrome causing effusion *(common)*
Advanced renal failure (primarily oliguric or anuric)

Dehydration (i.e., hypovolemia)
Gastrointestinal loss *(common)* (i.e., vomiting or diarrhea)
Third-space loss (i.e., pancreatitis, peritonitis, uroabdomen *[common]*, chylothorax with repeated pleural fluid drainage)
Cutaneous loss (i.e., burns)
Hypoadrenocorticism *(uncommon but important)*
Diuretic administration (including osmotic diuretics)

Normal hydration (i.e., normovolemia)
Inappropriate fluid therapy with 5% dextrose, 0.45% saline solution, or hypotonic fluids *(important)*
Psychogenic polydipsia
Syndrome of inappropriate antidiuretic hormone secretion (SIADH) *(rare)*
Antidiuretic drugs (e.g., heparin solutions containing chlorbutol, vincristine, cyclophosphamide, nonsteroidal antiinflammatory drugs)
Myxedema coma of hypothyroidism *(rare)*

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 60.

hyposthenuria, mild hyponatremia, and mild plasma hypoosmolality. SIADH refers to excessive antidiuretic hormone (ADH) release despite lack of normal stimuli; it can be caused by malignancy, pulmonary disease, or central nervous system (CNS) disorders. Diagnosis of SIADH requires eliminating adrenal, renal, cardiac, and hepatic disease and finding inappropriately high urine osmolality (> 100 mOsm/kg) despite serum hypoosmolality. Drugs that stimulate ADH release or potentiate its renal effects may lead to hyponatremia with normovolemia.

The most common causes of moderate to marked hyponatremia (i.e., Na < 135 mEq/L) in dogs and cats include vomiting, hypoadrenocorticism, and advanced congestive heart failure (with or without concomitant diuretic therapy). History or physical examination usually reveals the cause, but an ACTH-stimulation test should be performed if the clinician suspects hypoadrenocorticism. If the cause is still unknown, plasma osmolality measurements are recommended.

Causes of Hypernatremia • Hypernatremia is caused by loss of water, gain of sodium, or both (Box 6-4). It is rare for animals with normal thirst mechanisms and adequate access to water to become hypernatremic unless they are unable to ingest water.

Free-water loss (i.e., water without appreciable amounts of electrolytes) occurs in diabetes insipidus and insensible losses. Central diabetes insipidus (see Chapter 7) is due to lack of ADH production and release. Affected animals have severe polydipsia and polyuria, and hypernatremia is common. Nephrogenic diabetes insipidus is a category that includes many disorders characterized by renal urine concentration abnormalities (see Chapter 7). Insensible losses (i.e., normal respiratory tract losses) occur in all animals; if the patient cannot or will not drink, hypernatremia results (e.g., hypodipsia caused by an abnormal CNS thirst mechanism in young female miniature schnauzers). Clinical signs include anorexia, lethargy, weakness, disorientation, ataxia, and seizures.

Hypotonic water loss is loss of both water and electrolytes but more water than sodium. Such losses may be renal or extrarenal (e.g., gastrointestinal, third space, cutaneous). Vomiting, diarrhea, and small intestinal obstruction may cause hypotonic gastrointestinal losses. Third-space losses include pancreatitis and peritonitis. Cutaneous losses are rarely important in dogs and cats. Renal losses may result from lack of ADH, osmotic or drug-induced diuresis, or renal disease that affects concentrating ability.

BOX 6-4. Causes of Hypernatremia

Loss of Free Water *without Adequate Replacement* (important)
- Normal insensible water loss without normal replacement
- Water unavailable or patient unable to drink
- Abnormal thirst mechanism
  - Primary hypodipsia (e.g., miniature schnauzers) *(rare)*
  - Central nervous system (CNS) neoplasia
- Increased insensible water loss without replacement
- High environmental temperature, fever, tachypnea/panting
- Urinary loss of free water
- Diabetes insipidus (either central or nephrogenic)

Loss of Hypotonic Fluids *without Adequate Replacement of Water* (important)
- Extrarenal
  - Gastrointestinal (i.e., vomiting, diarrhea, small intestinal obstruction)
  - Third-space loss (i.e., peritonitis, pancreatitis)
  - Cutaneous (e.g., burns)
- Renal
  - Diuresis (osmotic [e.g., diabetes mellitus, mannitol], chemical [e.g., drugs])
  - Renal failure, postobstructive diuresis

Increased Intake of Sodium
- Hypertonic fluid administration (e.g., hypertonic saline, sodium bicarbonate, total parenteral nutrition solutions, sodium phosphate enema)
- Inappropriate maintenance fluid therapy with sodium-containing fluids (important)
- Salt poisoning
- Hyperaldosteronism *(rare)*
- Hyperadrenocorticism *(mild changes)*

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 53.

NOTE: If a patient with hypotonic fluid losses replaces fluids by drinking water, it may become *hypo*natremic instead of *hyper*natremic, because it is diluting remaining sodium with water it drinks.

Administration of excessive sodium (e.g., hypertonic saline, sodium bicarbonate, inappropriate fluid therapy) causes hypernatremia if the patient does not ingest adequate water. Hyperadrenocorticism, sodium phosphate enemas, and primary hyperaldosteronism (rare) may cause hypernatremia.

Clinically significant hypernatremia (i.e., Na > 160 mEq/L in dogs and 170 mEq/L

in cats) usually is due to a pure water deficit (e.g., unable to drink), loss of hypotonic fluid (e.g., gastrointestinal or renal losses), or fluid therapy. History is usually adequate to determine the cause of hypernatremia.

URINARY FRACTIONAL EXCRETION OF SODIUM

Seldom Indicated • Determining fractional sodium excretion (FE_{Na}) may help differentiate prerenal from primary renal azotemia (seldom needed for this purpose), and renal from extrarenal sodium loss in dehydrated patients with hypernatremia or hyponatremia. FE_{Na} is calculated by using the equation:

$$[(U_{Na}/S_{Na})/(U_{Cr}/S_{Cr})] \times 100$$

where

U_{Na} = urine concentration of sodium (mEq/L)

S_{Na} = serum concentration of sodium (mEq/L)

U_{Cr} = urine concentration of creatinine (mg/dl)

S_{Cr} = serum concentration of creatinine (mg/dl)

Normal Values • FE_{Na} should be less than 1% in normal dogs and cats.

Abnormalities • FE_{Na} should be less than 1% in animals with prerenal azotemia; greater than 1% suggests primary renal azotemia. Prerenal azotemia with FE_{Na} greater than 1% may occur despite normal renal function if the animal is receiving diuretics (e.g., furosemide). In dehydrated patients, FE_{Na} values less than 1% suggest nonrenal losses (e.g., gastrointestinal, third space); values greater than 1% suggest renal losses (e.g., hypoadrenocorticism, diuretic administration, renal disease).

SERUM CHLORIDE CONCENTRATION

Commonly Indicated • Serum chloride concentration commonly is measured in systemic diseases characterized by vomiting, diarrhea, dehydration, polyuria, and polydipsia or in patients likely to have metabolic acid-base abnormalities.

Analysis • Measured in serum, plasma, or urine by dry reagent systems, colorimetric titration, spectrophotometry (i.e., autoanalyzers), ion-specific potentiometry, and coulometric and amperometric titration. Results obtained by using point-of-care instruments do not always correlate well with results obtained by traditional methods.

Normal Values • Changes in water balance change chloride and sodium concentration proportionately. Chloride concentration can also change primarily; therefore evaluation of chloride concentration must be done in conjunction with evaluation of sodium concentration. Using this approach, chloride disorders can be divided into artifactual (sodium and chloride change proportionately) and corrected (changes in chloride are proportionately greater than changes in sodium) categories (Boxes 6-5 and 6-6). Changes in free water are responsible for the chloride changes in artifactual disorders. In corrected chloride disorders, chloride ion itself changes. Corrected chloride can be estimated as:

$$[Cl^-]\text{corrected} = [Cl^-] \text{ measured} \times 146/[Na^+] \text{ measured (for dogs)}$$

$$[Cl^-]\text{corrected} = [Cl^-] \text{ measured} \times 156/[Na^+] \text{ measured (for cats)}$$

where

$[Cl^-]$ measured and $[Na^+]$ measured are the patient's serum chloride and sodium concentrations, respectively. The values 146 and 156 reflect the mean value for serum sodium concentrations in dogs and cats. Normal $[Cl^-]$ corrected is approximately 107 to 113 mEq/L

BOX 6-5. Causes of Hypochloremia

Artifactual (Dilutional)

Corrected Hypochloremia

Pseudohypochloremia (lipemic samples using titrimetric methods)
Excessive loss of chloride relative to sodium
- Vomiting of stomach contents *(common and important)*
- Therapy with thiazide or loop diuretics *(common and important)*
- Chronic respiratory acidosis
- Hyperadrenocorticism
- Exercise

Therapy with solutions containing high sodium concentration relative chloride
Sodium bicarbonate

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 78.

BOX 6-6. Causes of Hyperchloremia

Artifactual (Concentration)
Corrected Hyperchloremia
Pseudohyperchloremia
  Lipemic samples using colorimetric methods
  Potassium bromide therapy *(common and important)*
Excessive loss of sodium relative to chloride
  Small bowel diarrhea *(common and important)*
Excessive gain of chloride relative to sodium
  Therapy with chloride salts (e.g., NH_4Cl, KCl)
  Total parenteral nutrition
Fluid therapy (e.g., 0.9% NaCl, hypertonic saline, KCl-supplemented fluids)
  Salt poisoning
  Renal chloride retention
    Renal failure
    Renal tubular acidosis
    Hypoadrenocorticism
    Diabetes mellitus
    Chronic respiratory alkalosis
    Drugs
    Spironolactone
    Acetazolamide

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 79.

in dogs and 117 to 123 mEq/L in cats. These values may vary among laboratories and analyzers.

Danger Values • Unknown. Metabolic alkalosis and decreased ionized calcium concentration probably cause muscle twitching or seizures in hypochloremic animals, whereas clinical signs associated with hyperchloremia are probably caused by hyperosmolality.

Artifacts • Pseudohypochloremia results when chloride is measured in lipemic or markedly hyperproteinemic samples via techniques that are not ion-selective. Hyperviscosity may cause problems in machines that dilute samples before analysis. In lipemic samples, chloride concentration is underestimated by some titrimetric methods and overestimated by colorimetric methods. Halides (e.g., bromide, iodide) are measured as chloride, falsely increasing measurements (especially important in animals receiving potassium bromide as an anticonvulsant).

Drugs That May Alter Serum Chloride Concentration • Administration of NH_4Cl, KCl, physiologic saline solution (with or without KCl), hypertonic saline solution, or total parenteral nutrition solutions containing arginine HCl and lysine HCl may add excessive Cl to the body. Acetazolamide may cause renal chloride retention. Hypochloremia may be caused by excessive renal loss of chloride relative to sodium (e.g., furosemide, thiazides) or excessive intake of sodium without chloride (e.g., $NaHCO_3$).

Causes of Hypochloremia • Many causes of hyponatremia also produce hypochloremia. If changes in sodium are proportional to changes in chloride (hypochloremia with normal corrected chloride or artifactual hypochloremia), it is usually easier to search for the cause of the hyponatremia. Corrected hypochloremia results from excessive loss of chloride relative to sodium or administration of fluids containing high sodium concentration relative to chloride (see Box 6-5). The most common causes of corrected hypochloremia are chronic vomiting of gastric contents and aggressive furosemide or thiazide therapy. Administration of sodium without chloride (e.g., $NaHCO_3$) also may cause corrected hypochloremia. Hypochloremia caused by increased renal chloride excretion is a normal adaptation to chronic respiratory acidosis. Persistent hypochloremia is an indication to determine serum sodium, potassium, and total carbon dioxide (Tco_2) concentrations (preferably by blood gas analysis).

Causes of Hyperchloremia • Most causes of hypernatremia produce concurrent hyperchloremia. If changes in sodium are proportional to changes in chloride (hyperchloremia with normal corrected chloride concentration or "artifactual" hyperchloremia) it usually is easier to search for the cause of the hypernatremia. Corrected hyperchloremia results from excessive sodium loss relative to chloride, excessive chloride gain relative to sodium, or renal chloride retention (see Box 6-6). Small bowel diarrhea can cause hyperchloremic metabolic acidosis because of loss of bicarbonate-rich, chloride-poor fluid (i.e., excessive sodium loss). Salt poisoning or therapy with NH_4Cl, KCl, cationic amino acids, hypertonic saline, or 0.9% NaCl with or without added KCl represent excessive chloride gain (e.g., physiologic saline solution has 154 mEq chloride/L but contains 174 mEq chloride/L if supplemented with 20 mEq KCl/L).

The most common cause of hyperchloremia is hypotonic fluid loss leading to

hyperchloremic (normal anion gap) metabolic acidosis. Persistent hyperchloremia is an indication for determining serum sodium, potassium, and Tco_2 concentrations and blood gas analysis.

OSMOLALITY AND OSMOLAL GAP

Osmolality refers to the number of osmotically active particles in a solution. *Tonicity* describes the osmolality of a solution relative to plasma. A solution with the same osmolality as plasma is said to be *isotonic*, whereas one with greater osmolality than plasma is *hypertonic*. A solution with osmolality lower than that of plasma is *hypotonic*. Tonicity depends on the ability of these particles to exert oncotic pressure and whether or not the particles can rapidly cross a semipermeable membrane (e.g., a cell membrane). For example, urea does not cause hypertonicity (i.e., exert oncotic pressure), because it rapidly diffuses across cell membranes and equilibrates throughout the body. Sodium and glucose cannot rapidly cross membranes; therefore they tend to stay on one side and cause hypertonicity (i.e., exert oncotic pressure), attracting fluids. Everything that affects tonicity (e.g., sodium) also affects osmolality, but not everything that affects osmolality also affects tonicity (e.g., urea).

Occasionally Indicated • Serum or plasma osmolality helps differentiate causes of hyponatremia, aids in early diagnosis of ethylene glycol intoxication, evaluates hydration status and renal concentrating ability during water deprivation testing, and sometimes helps evaluate patients with diabetic ketoacidosis and those being treated with mannitol for cerebral edema.

Disadvantage • Special equipment (e.g., freezing point depression or vapor pressure osmometer) is required.

Analysis • Osmolality is *measured* in serum, plasma, or urine by freezing point or vapor pressure osmometry (citrate anticoagulants cause artifactual increases). It is estimated (i.e., *calculated*) by various formulas. In the absence of excessive unmeasured osmoles (e.g., ethylene glycol), the following formula closely estimates osmolality:

$$\text{Osmolality (mOsm/kg)} = 1.86([Na^+] + [K^+]) + \text{glucose}/18 + \text{BUN}/2.8 + 9$$

Where serum sodium and potassium are expressed in mEq/L and BUN and glucose in mg/dl. However, $2 \times [Na]$ may be used as a quick estimate of osmolality. Tonicity may be estimated by the following formula:

$$\text{Tonicity} = \text{Plasma osmolality} - \text{BUN}/2.8$$

Where tonicity and osmolality are expressed in mOsm/kg and BUN is expressed in mg/dl.

Normal Values • Serum or plasma osmolality: dogs, 290 to 310 mOsm/kg; cats, 308 to 335 mOsm/kg. Urine osmolality values vary widely. Typical ranges are 50 to 2800 mOsm/kg (dogs) and 50 to 3000 mOsm/kg (cats).

Danger Values • Signs because of hypoosmolality or hyperosmolality are related more to rapidity of change than magnitude of change. Neurologic signs (e.g., disorientation, ataxia, seizures, coma) may occur when serum or plasma osmolality is less than 250 mOsm/kg or tonicity is greater than 360 mOsm/kg.

Osmolal gap is defined as measured serum osmolality – calculated serum osmolality. An increased gap is due to unmeasured osmoles (e.g., ethylene glycol metabolites), pseudohyponatremia (i.e., normal osmolality plus hyponatremia), or laboratory error. Vapor pressure osmometry does not detect volatile solutes (e.g., methanol). If measured osmolality is less than calculated osmolality, a laboratory error is probably responsible.

Normal Values • Dogs, 10 to 15 mOsm/kg; cats, unknown.

Danger Values • Osmolal gap greater than 25 mOsm/kg indicates the presence of an unmeasured osmole, usually as a result of intoxication (e.g., ethylene glycol, methanol, ethanol).

Causes of Serum or Plasma Hypoosmolality • See Hyponatremia.

Causes of Serum or Plasma Hypoosmolality • Hypernatremia, hyperglycemia, severe azotemia, glycerin, and intoxications (e.g., ethylene glycol, ethanol, methanol). The most common causes of serum osmolality greater than 360 mOsm/kg are diabetic ketoacidosis, azotemia, and hypernatremia. Citrate anticoagulants may cause increased readings. Hyperosmolality is an indication to measure serum sodium, potassium, urea

nitrogen, and glucose concentrations plus calculate anion and osmolal gaps.

Causes of Increased Osmolal Gap • Pseudohyponatremia, glycerin, ethylene glycol, methanol, ethanol, and possibly other intoxications increase the osmolal gap. Mannitol or lactic acid might also be responsible. If pseudohyponatremia is ruled out, an increased osmolal gap mandates a search for recent exposure to these toxins. The increase in osmolal gap in dogs with ethylene glycol intoxication peaks at 6 hours, persists for at least 12 hours, but may be normal 24 hours after ingestion. If ethylene glycol intoxication is likely, urinalysis looking for calcium oxalate crystals, blood gas analysis, anion gap, and appropriate toxicologic analyses (see Chapter 17) are indicated.

BLOOD GAS ANALYSIS

Occasionally Indicated • Acid-base evaluation is useful in severely ill pets (e.g., severe dehydration, vomiting, diarrhea, oliguria and anuria, hyperkalemia, tachypnea). Blood gas analysis is also necessary to evaluate gas exchange and Tco_2 alterations in patients with respiratory disorders (see Chapter 11). Urine pH does not necessarily reflect systemic pH and cannot substitute for blood gas analysis.

Advantages • Blood gas analysis allows precise identification of the different acid-base disturbances and aids in evaluation of pulmonary function.

Disadvantages • Equipment is expensive, and careful technique is required in obtaining and handling blood specimens to prevent artifacts. The need for rapid analysis may prohibit use of remote laboratories; however, point-of-care units (e.g., immediate response mobile analysis [IRMA]; see Chapter 1) allow immediate determinations and are often affordable for busy practices.

Analysis • Blood gas analyzers are equipped with specific electrodes to measure pH, carbon dioxide tension (P_{CO_2}), and oxygen tension (P_{O_2}). The HCO_3^- is calculated. Arterial blood is required to evaluate P_{O_2} for pulmonary function, but free-flowing jugular blood is acceptable for acid-base analysis. Pulmonary artery, jugular vein, and cephalic vein samples usually have similar values in normal dogs, whereas arterial blood has a slightly lower HCO_3^- (21 mEq/L versus 22 to 23 mEq/L for venous blood) and much lower P_{CO_2} (37 mm Hg versus 42 to 43 mm Hg for venous blood). Abnormal cardiovascular function may change this relationship.

For routine blood gas analyzers, a 3 ml syringe with a 25-gauge needle is used to collect 0.5 to 1.5 ml of blood. Heparin (1000 U/ml) is drawn into the syringe (coating the interior) and it and all air are expelled, leaving the needle hub filled with heparin (approximately 0.1 to 0.2 ml). For point-of-care units, as little as 0.125 ml blood is required. After the blood is collected, air bubbles must be dislodged and expelled. Inserting the needle into a rubber stopper or placing a tightly fitting cap over the syringe hub prevents exposure of the sample to room air. The syringe is rolled between the palms of the hands to mix the sample, then syringe and blood are submitted. Analysis should occur within 15 to 30 minutes of collection (if stored at 25° C) or within 2 hours if the sample is immersed in an ice water bath. Handheld devices (e.g., IRMA) developed for use at the bedside (or cage) have been marketed as point-of-care units. These units can provide rapid blood gas data (as well as electrolytes and selected other determinations) on critically ill patients that can aid in decision making while waiting for routine laboratory results. There appears to be good correlation between results obtained with these small units and those coming from larger laboratory units. See Chapter 1 for a comparison of the IRMA unit and the larger, traditional blood gas analyzers relative to accuracy and cost effectiveness.

Normal Values • Normal blood gas values are shown in Table 6-1.

Danger Values • pH less than 7.10 indicates life-threatening acidosis, which may impair myocardial contractility; pH greater than 7.60 denotes severe alkalosis.

Artifacts • P_{CO_2} decreases, whereas pH and P_{O_2} increase if the sample is exposed to air. Air bubbles in the sample may do the same, especially if they occupy greater than or equal to 10% of sample volume. P_{CO_2} increases and pH decreases if analysis is delayed. Aerobic metabolism by WBCs may decrease P_{O_2}. Cooling the sample from 25° to 4° C slows

TABLE 6-1. Normal Blood Gas Values

	PH	P_{CO_2} (mm Hg)	HCO_3 (mEq/L)	P_{O_2} (mm Hg)
Dog venous	7.32-7.40	33-50	18-26	
Dog arterial	7.36-7.44	36-44	18-26	≈100
Cat venous	7.28-7.41	33-45	18-23	
Cat arterial	7.36-7.44	28-32	17-22	≈100

these changes. Prolonged venous stasis during venipuncture increases P_{CO_2} and decreases pH. Excessive heparin (>10% of the sample volume) decreases pH, P_{CO_2}, and HCO_3^-, whereas citrate, oxalate, or EDTA may decrease pH. Blood gas analyzers calculate HCO_3^- from pH and P_{CO_2}; T_{CO_2} is measured on serum chemistry autoanalyzers but calculated on many blood gas analyzers. T_{CO_2} usually is 1 to 2 mEq/L higher than HCO_3^-.

Drugs That May Alter Blood Gas Results • Acetazolamide, NH_4Cl, and $CaCl_2$ may cause acidosis. Antacids, sodium bicarbonate, potassium citrate or gluconate, and loop diuretics may cause alkalosis. Salicylates may cause metabolic acidosis, respiratory alkalosis, or both.

Analysis of Blood Gas Results • The clinician should begin by evaluating the pH. If it is abnormal, an acid-base disturbance exists. If the pH is within the normal range, the clinician should check the P_{CO_2} and HCO_3^-. If they are abnormal, a mixed acid-base disturbance is probably present. If the pH is low and the HCO_3^- decreased, *metabolic acidosis* is present. If the pH is low and P_{CO_2} increased, *respiratory acidosis* is present. If the pH is high and the HCO_3^- increased, *metabolic alkalosis* is present. If the pH is high and P_{CO_2} decreased, *respiratory alkalosis* is present.

Next, the clinician should calculate the expected compensatory response (e.g., respiratory alkalosis is compensation for metabolic acidosis; metabolic alkalosis is compensation for respiratory acidosis) using the guidelines in Table 6-2. These guidelines are for dogs only. If a patient's compensatory response is within the expected range (i.e., within 2 mm Hg or 2 mEq/L of the calculated values), the acid-base disturbance is *simple*. If the compensatory response falls outside of the expected range, more than one acid-base disorder (i.e., a *mixed* disorder) is present.

After classifying the type of disturbance and whether it is simple or mixed, the clinician should determine whether the acid-base disturbance is compatible with the patient's history and clinical findings. If the acid-base disturbance does not fit with the patient's history, clinical findings, and other laboratory data, the blood gas analysis should be questioned.

Metabolic Acidosis • Metabolic acidosis (i.e., decreased pH and HCO_3^-, with a compensatory decrease in P_{CO_2}) is caused by addition of acid, failure to excrete acid, loss of HCO_3^-, or a combination thereof (Box 6-7). Addition of acid may be iatrogenic (e.g., ethylene glycol, salicylates, NH_4Cl, cationic amino acids) or spontaneous (i.e., lactic acidosis, ketoacidosis). Decreased acid excretion is due to renal dysfunction (e.g., renal failure, hypoadrenocorticism, type I renal tubular acidosis [RTA]). Loss of HCO_3^- is usually caused by small bowel diarrhea (i.e., diarrheic fluid has more HCO_3^- than plasma); renal losses of HCO_3^- (e.g., carbonic anhydrase inhibitors, type II RTA) are rare. Metabolic acidosis is usually caused by renal failure, diabetic ketoacidosis, lactic acidosis from poor perfusion, hypoadrenocorticism, and perhaps small bowel diarrhea. Anion gap sometimes helps differentiate these causes and is discussed later. Measurement of blood lactate concentrations may help determine the cause of the acidosis and may also be prognostic (i.e., increased blood lactate is associated with a poorer prognosis). See Chapter 14 for a brief discussion of blood lactate measurement.

Respiratory Acidosis • Respiratory acidosis (i.e., decreased pH, increased P_{CO_2}, with a compensatory increase in HCO_3^-) is due to hypoventilation (which increases P_{CO_2}) and is synonymous with "primary hypercapnia" (Box 6-8). Hypoventilation may be caused by airway obstruction, cardiopulmonary arrest, restrictive respiratory diseases (e.g., diaphragmatic hernia, pneumothorax, pleural effusion, hemothorax, chest wall trauma, pulmonary fibrosis, pyothorax), severe pulmonary diseases,

TABLE 6-2. Renal and Respiratory Compensations for Primary Acid-Base Disorders in Dogs

DISORDER	PRIMARY CHANGE	COMPENSATORY RESPONSE
Metabolic acidosis	↓ $[HCO_3^-]$	0.7 mm Hg decrement in P_{CO_2} for each 1 mEq/L decrement in $[HCO_3^-]$
Metabolic alkalosis	↑$[HCO_3^-]$	0.7 mm Hg increment in P_{CO_2} for each 1 mEq/L increment in $[HCO_3^-]$
Acute respiratory acidosis	↑P_{CO_2}	1.5 mEq/L increment in $[HCO_3^-]$ for each 10 mm Hg increment in P_{CO_2}
Chronic respiratory acidosis	↑ P_{CO_2}	3.5 mEq/L increment in $[HCO_3^-]$ for each 10 mm Hg increment in P_{CO_2}
Acute respiratory alkalosis	↓P_{CO_2}	2.5 mEq/L decrement in $[HCO_3^-]$ for each 10 mm Hg decrement in P_{CO_2}
Chronic respiratory alkalosis	↓P_{CO_2}	5.5 mEq/L decrement in $[HCO_3^-]$ for each 10 mm Hg decrement in P_{CO_2}

From DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 196.

and inadequate mechanical ventilation. Hypoventilation may also result from respiratory paralysis from neuromuscular disease (e.g., myasthenia gravis, tetanus, botulism, polyradiculoneuritis, tick paralysis, hypokalemic polymyopathy), as well as by neuromuscular blocking drugs (e.g., succinylcholine, pancuronium, aminoglycosides combined with anesthetics). Airway obstruction, cardiac arrest, and respiratory paralysis usually cause respiratory acidosis. These patients are invariably hypoxemic if breathing room air. Breathing oxygen-enriched air (i.e., anesthesia) sometimes causes normal or increased P_{O_2}.

BOX 6-7. Causes of Metabolic Acidosis

Increased Anion Gap (Normochloremic)
Ethylene glycol intoxication *(important)*
Diabetic ketoacidosis* *(common and important)*
Uremic acidosis† *(common and important)*
Lactic acidosis *(common and important)*
Salicylate intoxication
Other rare intoxications (e.g., paraldehyde, methanol)

Normal Anion Gap (Hyperchloremic)
Hypoadrenocorticism‡ *(important)*
Diarrhea
Carbonic anhydrase inhibitors (e.g., acetazolamide)
Dilutional acidosis (e.g., rapid administration of 0.9% saline)
Ammonium chloride *(infrequent)*
Cationic amino acids (e.g., lysine, arginine, histidine) *(rare)*
Posthypocapnic metabolic acidosis *(rare)*
Renal tubular acidosis (RTA) *(rare)*

*Patients with diabetic ketoacidosis may have some component of hyperchloremic metabolic acidosis in conjunction with increased anion gap acidosis.
†The metabolic acidosis early in renal failure may be hyperchloremic and later convert to increased anion gap acidosis.
‡Patients with hypoadrenocorticism typically have hypochloremia caused by impaired water excretion (dilutional effect) and absence of aldosterone.
Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 213.

BOX 6-8. Causes of Respiratory Acidosis

Airway Obstruction
Aspiration (e.g., foreign body, vomitus)

Respiratory Center Depression
Neurologic disease (e.g., brain stem, high cervical spinal cord lesion)
Drugs (e.g., narcotics, sedatives, barbiturates, inhalation anesthetics)
Toxemia

Cardiopulmonary Arrest *(common)*

Neuromuscular Defects
Myasthenia gravis, tetanus, botulism, polyradiculoneuritis, polymyositis, tick paralysis, hypokalemic periodic paralysis in Burmese, hypokalemic myopathy in cats
Drug-induced (i.e., succinylcholine, pancuronium, aminoglycosides with anesthetics, organophosphates)

Restrictive Diseases
Diaphragmatic hernia, pneumothorax, pleural effusion, hemothorax, pyothorax, chest wall trauma, pulmonary fibrosis

Pulmonary Disease *(less common)*
Respiratory distress syndrome, pneumonia, severe pulmonary edema, diffuse metastatic disease, smoke inhalation, pulmonary thromboembolism, chronic obstructive pulmonary disease, pulmonary mechanical ventilation fibrosis

Inadequate Ventilation

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 246.

Metabolic Alkalosis • Metabolic alkalosis (i.e., increased pH and HCO_3^-, with a compensatory increase in P_{CO_2}) is caused by loss of acid from or addition of alkali to the body (Box 6-9). Loss of acid usually is due to vomiting gastric fluid, but loss of Cl^- via the kidneys (i.e., caused by furosemide) may be responsible. Metabolic alkalosis is usually due to vomiting of gastric contents (especially but not exclusively because of gastric outflow obstruction) or administration of furosemide. Adding alkali may occur by administering $NaHCO_3$, lactated Ringer's solution, or potassium citrate. Normal kidneys eliminate administered alkali, however, and iatrogenic alkalosis seldom results unless renal dysfunction is present.

Respiratory Alkalosis • Respiratory alkalosis (i.e., increased pH, decreased P_{CO_2}, with a compensatory decrease in HCO_3^-) is due to tachypnea (which decreases P_{CO_2}) and is synonymous with "primary hypocapnia" (Box 6-10). It may be the result of pulmonary disease, pulmonary thromboembolism, hypoxemia, direct stimulation of the medullary respiratory center (e.g., gram-negative sepsis, hepatic disease, salicylates, xanthines, CNS disease, heat stroke), and excessive mechanical ventilation. Unexplained respiratory alkalosis may suggest gram-negative sepsis or pain. Pulmonary edema may cause respiratory alkalosis, metabolic acidosis, or respiratory acidosis. Respiratory alkalosis may occur during recovery from metabolic acidosis because hyperventilation (the compensation for metabolic acidosis) persists for 24 to 48 hours after correction of the acidosis. These patients are sometimes hypoxemic. Respiratory disease sometimes initially causes tachypnea and consequently hypocapnia, which can change to hypercapnia if the disease worsens.

BOX 6-9. Causes of Metabolic Alkalosis

Vomiting of gastric contents *(common and important)*
Diuretic therapy (e.g., loop diuretics, thiazides) *(important)*
Oral administration of sodium bicarbonate or other organic anions (e.g., lactate, citrate, gluconate, acetate)
Hyperadrenocorticism *(infrequent)*
Posthypercapnia *(rare)*
Primary hyperaldosteronism *(rare)*

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 231.

BOX 6-10. Causes of Tachypnea Resulting in Respiratory Alkalosis

Hypoxemia from Almost Any Cause

Right-to-left shunting, decreased P_IO_2 (e.g., residence at high altitude), congestive heart failure, severe anemia, pulmonary disease

Central Nervous System (CNS) (direct stimulation of medullary respiratory center)

Central neurologic disease, hepatic disease, gram-negative sepsis, drugs (i.e., salicylate intoxication, xanthines such as aminophylline), heat stroke, fear, pain, fever, hyperthyroidism

Mechanical Ventilation

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 247.

TOTAL CARBON DIOXIDE FOR ACID-BASE EVALUATION

T_{CO_2} is synonymous with HCO_3^- in samples handled aerobically.

Frequently Indicated • In any severe systemic disease process, T_{CO_2} helps determine if blood gas analysis is needed. Diseases in which T_{CO_2} determinations and blood gas analysis are indicated include ethylene glycol or salicylate intoxication, severe diabetic ketoacidosis, and severe uremia.

Advantage • Abnormal T_{CO_2} may be an indication to obtain blood gas analysis.

Disadvantages • One cannot accurately define metabolic and respiratory acid-base disorders using just T_{CO_2}. High T_{CO_2} could be the result of either metabolic alkalosis or compensated respiratory acidosis. Low T_{CO_2} could be the result of either metabolic acidosis or compensated respiratory alkalosis.

Analysis • T_{CO_2} is measured in serum or plasma by enzymatic and dry reagent methods. Serum or plasma analyzed within 15 to 20 minutes of collection is preferred. Samples may be stored in a capped syringe on ice at 4° C for up to 2 hours before analysis.

Normal Values • Dogs and cats, 17 to 23 mEq/L.

Danger Values • Less than 12 mEq/L (implies but does not confirm diagnosis of severe metabolic acidosis).

Artifacts • Tco_2 determined by dry reagent methods is not affected by hyperlipidemia. Falsely decreased Tco_2 occurs if processing the blood sample is delayed for several hours, if the blood collection tube is underfilled, and if heparin anticoagulant occupies greater than 10% of the sample volume.

Drugs That May Alter Tco_2 • Acetazolamide and NH_4Cl cause metabolic acidosis, reducing Tco_2. Furosemide, thiazides, and sodium bicarbonate cause metabolic alkalosis, increasing Tco_2.

Causes of Decreased Tco_2 • Tco_2 concentrations are decreased in metabolic acidosis (most common cause) and compensated respiratory alkalosis. A hyperventilating animal with decreased Tco_2 usually has metabolic acidosis but could have chronic respiratory alkalosis. Blood gas analysis may be necessary to determine which is present. Severely decreased Tco_2 in a patient with a recognized cause of metabolic acidosis (e.g., diabetic ketoacidosis) is usually assumed to represent metabolic acidosis. Blood gas analysis is necessary to confirm the presence of metabolic acidosis and assess the severity of the change in pH. Tco_2 less than or equal to 12 mEq/L in a patient with undiagnosed systemic disease is an indication for blood gas analysis. If blood gas analysis is unavailable, the clinician must correlate Tco_2 with the clinical setting and decide if $NaHCO_3$ therapy is indicated. This approach however can be dangerous because the actual pH is not known. Measuring serum electrolyte concentrations allows optimal fluid therapy (i.e., disturbances that affect acid-base balance often also cause electrolyte abnormalities).

Causes of Increased Tco_2 • Tco_2 concentrations are increased in metabolic alkalosis (most common) and compensated respiratory acidosis. Serum sodium, potassium, and chloride concentrations should be measured, because hypochloremia and hypokalemia are common in metabolic alkalosis. If these changes occur, they should be corrected (i.e., administration of 0.9% NaCl + KCl) and the underlying cause diagnosed (e.g., pyloric obstruction).

ANION GAP

Infrequently Indicated • Anion gap sometimes helps differentiate causes of metabolic acidosis and may help clarify mixed acid-base disorders. Metabolic acidosis with a high anion gap usually comes from acids that do not contain chloride (e.g., lactic acid, keto acids, salicylic acid, metabolites of ethylene glycol, phosphates, sulfates). Metabolic acidosis characterized by a normal anion gap has an increased plasma chloride concentration and is called hyperchloremic acidosis.

Advantage • Only a simple calculation from values already measured is required.

Disadvantage • Anion gap is affected by several factors and can be difficult to interpret.

Analysis • The anion gap is calculated as $(Na^+ + K^+) - (Cl^- + HCO_3^-)$ or $Na^+ - (Cl^- + HCO_3^-)$, depending on the clinician or laboratory's preference. The anion gap and its component values are expressed in mEq/L. If the patient is severely hypoalbuminemic, the anion gap may not reflect expected findings. For each 1 g/dl decrease in serum albumin, the anion gap decreases approximately 2.4 mEq/L.

Normal Values • Normal anion gap calculated by $(Na^+ + K^+) - (Cl^- + HCO_3^-)$ is approximately 12 to 24 mEq/L in dogs and 13 to 27 mEq/L in cats.

Danger Values • Greatly increased values may be the result of acute ethylene glycol intoxication and warrant a careful review of the patient's history. There may be a correlation between increasing anion gap and mortality in seriously ill animals.

Causes of Decreased Anion Gap • Hypoalbuminemia is probably the most common cause of a decreased anion gap; IgG multiple myeloma may also be responsible. The magnitude of increase in unmeasured cations (e.g., calcium, magnesium) necessary to lower the anion gap would probably be fatal. Laboratory errors resulting in overestimation of Tco_2 or Cl^- or in underestimation of sodium may artifactually decrease the anion gap. A decreased anion gap is seldom clinically significant.

Causes of Normochloremic (Increased Anion Gap) Acidosis • The most common causes of an increased anion gap in acidotic patients are lactic acidosis, diabetic

ketoacidosis, uremic acidosis, ethylene glycol intoxication, and laboratory error.

Causes of Hyperchloremic (Normal Anion Gap) Acidosis • Severe, acute small bowel diarrhea causes HCO_3^- loss and produces hyperchloremic (normal anion gap) acidosis. Carbonic anhydrase inhibitors (e.g., acetazolamide) inhibit proximal renal tubular reabsorption of HCO_3^- and produce self-limiting hyperchloremic metabolic acidosis. Acidosis because of administration of NH_4Cl decreases HCO_3^-, but serum Cl^- increases and anion gap is unchanged. Infusion of cationic amino acids (e.g., lysine HCl, arginine HCl) during total parenteral nutrition may cause hyperchloremic metabolic acidosis, because H^+ ions are released when urea is generated. Renal acid excretion decreases during chronic respiratory alkalosis, with consequent reduction in plasma HCO_3^- and increase in Cl^-. When the stimulus for hyperventilation is removed and P_{CO_2} increases, pH decreases because it requires 1 to 3 days for the kidneys to increase acid excretion and increase plasma HCO_3^-. This transient phenomenon is called *posthypocapnic metabolic acidosis* and is associated with hyperchloremia.

Dilutional acidosis occurs when extracellular volume is expanded via an alkali-free chloride-containing solution (e.g., 0.9% NaCl). The high Cl^- of physiologic saline solution (i.e., 154 mEq/L) and the highly resorbable nature of Cl^- in renal tubules contribute to decreased plasma HCO_3^- and hyperchloremia. RTA is a rare disorder characterized by hyperchloremic metabolic acidosis because of either decreased HCO_3^- reabsorption (type II RTA) or defective acid excretion (type I RTA).

Other Causes of Increased Anion Gap • Severe dehydration may increase both serum albumin concentration and the anion gap. Alkalemia may increase anion gap slightly. Excessive standing of serum, especially in uncapped containers, also may increase anion gap (a common error in samples not analyzed until the next day).

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7

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Urinary Disorders

DIFFERENTIATION OF POLYURIA-POLYDIPSIA, DYSURIA, AND INCONTINENCE

These three abnormalities must be differentiated early in the diagnostic workup by history or direct observation of urination. *Polyuria-polydipsia* (pu-pd) means urine production and water consumption in excess of normal. *Dysuria* means difficult urination and implies unduly frequent urination with small or minimal amounts voided each time. Clients may assume that increased frequency means increased volume. The diagnostician must ascertain whether the amount of urine voided with each micturition is large, small, or unknown. Animals with pu-pd and dysuria are conscious of their voiding and do not wake up in puddles of urine, unless they are too weak or in too much pain to get up. Patients with urinary *incontinence* may wake up soaked in urine, leave a spot of urine where they slept, or dribble urine as they walk or run.

Polyuria-Polydipsia

A precise history and consistently low urine specific gravities (i.e., < 1.030) suggest pu-pd. Measurement of total water consumption (including water in food) over at least a 24-hour period is necessary to verify polydipsia (normal water intake is 20 to 70 ml/kg/day). This measurement is best performed at the client's home because some polyuric animals will not readily drink water at a clinic. Quantitation of urine production is difficult

unless a metabolism cage is available (normal 20 to 45 ml/kg/day).

Pu-pd has many possible causes (Table 7-1). History and physical examination are crucial in evaluating patients with pu-pd (Figure 7-1). Iatrogenic causes must be sought from the history (e.g., diuretics, glucocorticoids, anticonvulsants, high-salt or very-low–protein diets, excessive thyroid supplementation). Aminoglycosides tend to produce polyuric, acute renal failure. Glucocorticoids can cause pu-pd, even when administered rectally or topically. Pyometra is usually suggested by history (i.e., recent estrus) or physical examination findings (e.g., enlarged uterus, vaginal discharge). If the clinician is unsure whether pyometra is likely, complete blood count (CBC) and abdominal imaging are usually definitive (i.e., neutrophilic leukocytosis with left shift and enlarged uterus). Weight loss plus pu-pd in a cat suggests hyperthyroidism, renal failure, or diabetes mellitus (hyperthyroidism is rare in dogs). Enlarged thyroid glands may be palpated in the neck. Feline kidneys are usually palpable; size and contour should be assessed. Postoliguric diuresis is usually diagnosed from the history (e.g., a male cat that has undergone removal of a urethral obstruction). Pu-pd is the most common presenting complaint of hyperadrenal dogs; most affected dogs have a

TABLE 7-1. Causes of Polyuria-Polydipsia (pu-pd) in Dogs and Cats

CAUSES	REMARKS
Iatrogenic; drugs (e.g., diuretics, corticosteroids, thyroxin, anticonvulsants, aminoglycosides, or amphotericin B); salty or very-low–protein diets	History is informative BUN should be low with low protein diets
Renal disease	Urine specific gravity should be persistently 1.008-1.029 (dogs) May be azotemic or nonazotemic For nonazotemic renal failure, creatinine clearance is useful for diagnosis of renal dysfunction as the cause
Upper urinary tract infection	May be hyposthenuric Excretory urography is diagnostic test of choice, but ultrasound may be helpful
Fanconi's syndrome	Usually nonazotemic, hyperchloremic acidosis, glucosuric, and aminoaciduric
Diabetes mellitus	Hyperglycemic *Note:* Cats are prone to stress-induced hyperglycemia; urine glucose measured at the same time is usually (but not always) negative
Central diabetes insipidus	Hyposthenuric when euhydrated (see Figure 7-7)
Nephrogenic diabetes insipidus	Hyposthenuric when euhydrated May be primary (congenital), idiopathic, or secondary (pyelonephritis, hyperadrenocorticism,hypercalcemia, hypokalemia, pyometra, prostatic abscessation, *E. coli* septicemia, hypoadrenocorticism)
Hyperadrenocorticism	Isosthenuric, hyposthenuric, or concentrated urine Common cause of pu-pd in old dogs, rare in cats
Hypoadrenocorticism	Pu-pd occurs in approximately 20% of patients; may closely resemble renal failure but differentiated by absence of a stress leukogram and presence of hyperkalemia despite polyuria; ACTH response test is necessary for confirmation
Hypercalcemia	Isosthenuric or hyposthenuric, azotemic or nonazotemic
Hepatic insufficiency	Isosthenuric, hyposthenuric, or concentrated; may resemble hyperadrenocorticism (hepatic enzymes may be increased), but can also have normal ALT and SAP
Hyperthyroidism	Primarily in older cats but may be iatrogenic because of supplementation
Hyponatremia	Loss of sodium from any cause May cause isosthenuria whenever <120 mEq/L
Posturethral obstruction	Occurs occasionally after removal of a urethral obstruction that has resulted in uremia
Hypokalemia	Must be persistent and severe to cause polyuria
Polycythemia vera	Rare
Apparent psychogenic polydipsia	Concentrate urine in response to water deprivation (see Figure 7-7)
Acromegaly	Rare

ACTH = adrenocorticotropic hormone; ALT = alanine aminotransferase; BUN = blood urea nitrogen; SAP = serum alkaline phosphatase.

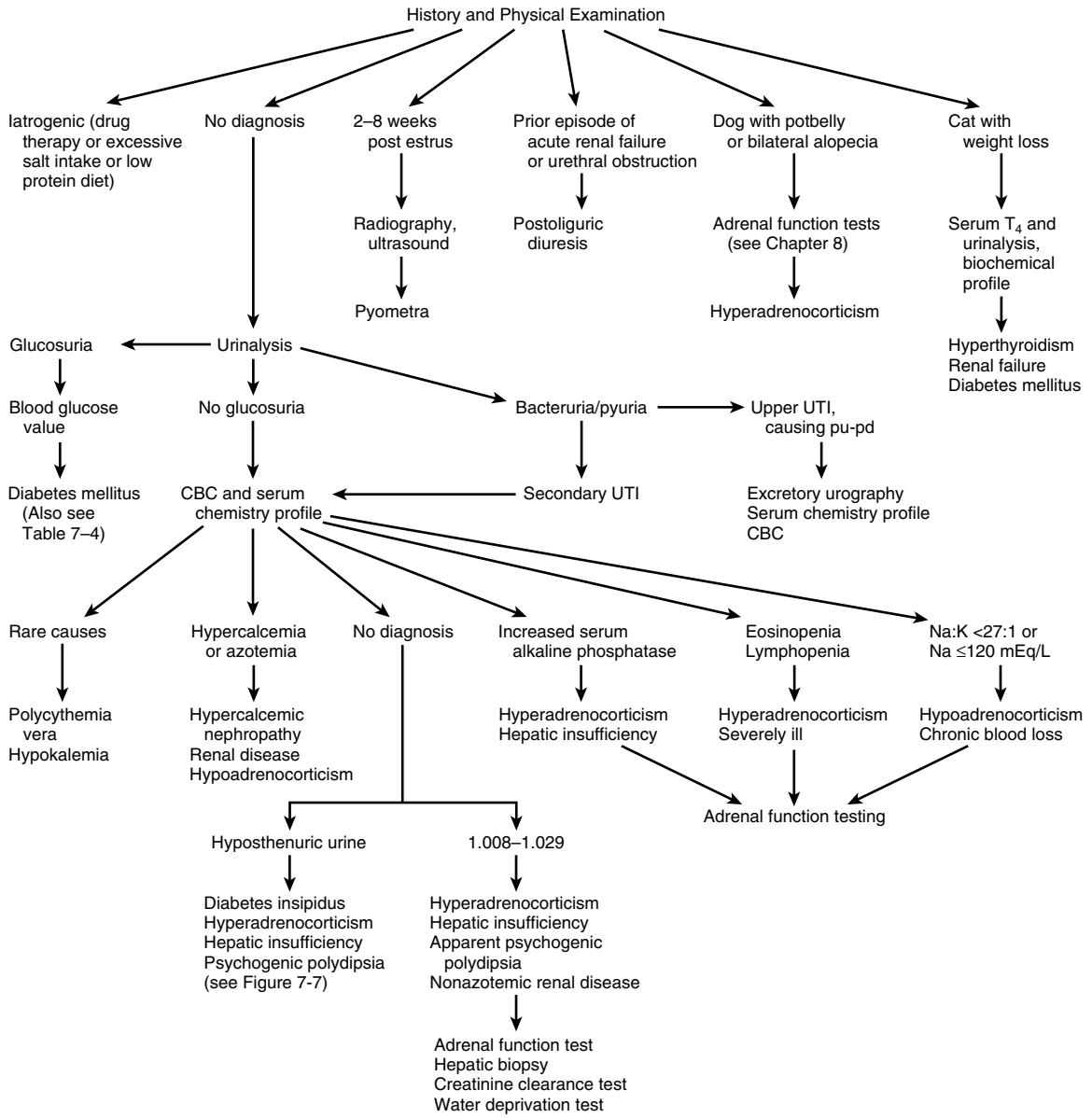

FIGURE 7-1. Diagnostic approach in a dog or cat with a history of polyuria-polydipsia (pu-pd); persistently hyposthenuric, isosthenuric, or inadequately concentrated urine; or a water balance study documenting water consumption >70 ml/kg/day. CBC = complete blood count; UTI = urinary tract infection.

pendulous abdomen, hepatomegaly, alopecia, or a combination thereof on physical examination. Such dogs should undergo adrenal function testing (see Chapter 8). Recent onset of cataracts suggests diabetes mellitus. Peripheral lymphadenopathy suggests lymphoma causing hypercalcemia. Other neoplasms (e.g., anal sac apocrine gland adenocarcinomas) may also cause hypercalcemia.

If the cause is not obvious, a urinalysis, CBC, and biochemical profile are the next steps (see Figure 7-1). Urinary tract infections (UTIs) may be secondary to hyperadrenocorticism or diabetes mellitus, but renal infection (i.e., bacterial pyelonephritis) can cause pu-pd. Neutrophilic leukocytosis, azotemia, white blood cell (WBC) casts, renal pain, or hyposthenuria may occur with pyelonephritis.

Pyelonephritis can be difficult to diagnose; excretory urography is the diagnostic method of choice (although abnormalities may also be found with ultrasonography if the renal pelvis can be imaged). Sometimes a presumptive diagnosis can only be made by response to long-term antibiotic therapy after failure of short-term therapy. Clinicopathologic screening often reveals changes indicative of the cause of pu-pd (e.g., renal failure, hyperadrenocorticism, hepatic insufficiency, hypercalcemia, diabetes mellitus) (see Figure 7-1). Serum thyroxine determinations are always indicated in old cats (i.e., > 10 years old).

Many dogs with hyperadrenocorticism are obviously cushingoid on physical examination (i.e., cutaneous abnormalities, potbellied appearance, hepatomegaly). The clinician does not find invariable CBC or serum chemistry profile changes; however, lymphopenia, eosinopenia, increased serum alkaline phosphatase (SAP), alanine aminotransferase (ALT), serum cholesterol, or a combination thereof is common. Approximately 40% to 50% of hyperadrenal dogs have UTI. Bacteriuria is often the only abnormality on urinalysis (i.e., no hematuria or pyuria). Adrenal function tests (see Chapter 8) or hepatic biopsy may be necessary to distinguish hyperadrenocorticism from primary hepatic disease.

Renal failure, hypercalcemic nephropathy, and hypoadrenocorticism can resemble each other. The first two usually produce pu-pd, whereas the last causes it in 15% to 25% of affected dogs. Each may have various degrees of azotemia, decreased renal concentrating ability (e.g., specific gravity 1.012 to 1.029), and hypercalcemia (10% to 15% of renal failure and 30% of hypoadrenal dogs). Most hypoadrenal patients have a serum Na:K of less than or equal to 27:1 with hyponatremia, hyperkalemia, or both. Classically, no stress leukogram exists despite the illness. An adrenocorticotropic hormone (ACTH) stimulation test (see Chapter 8) is needed to confirm the diagnosis, because other disorders may cause similar changes. Most azotemic renal failure patients are hyperphosphatemic and only mildly hypercalcemic (i.e., < 13 mg/dl), whereas most animals with hypercalcemia of nonrenal origin are normophosphatemic or mildly hypophosphatemic and may be markedly hypercalcemic. Nevertheless, distinguishing whether hypercalcemia is the cause or consequence of renal failure can be difficult if persistent hypercalcemia has produced renal damage plus hyperphosphatemia (especially when hypercalcemia is mild [11.5 to 14 mg/dl]). Such patients need a thorough search for neoplasia and may require measurement of ionized calcium and parathyroid hormone (PTH) concentrations (see Chapter 8). Most dogs with hypoadrenocorticism and primary renal failure have normal to decreased ionized calcium, whereas animals with primary hypercalcemic disorders (i.e., hyperparathyroidism, hypercalcemia of malignancy) have increased ionized calcium concentrations.

More extensive testing is necessary if the diagnosis is still uncertain (see Figure 7-1). Some dogs with renal failure are polyuric because of loss of functional nephron number (may occur with 67% reduction in renal function) but maintain a sufficient glomerular filtration rate (GFR) to avoid azotemia (which requires a 75% reduction in GFR). A creatinine or iohexol clearance test is a noninvasive way to identify these patients. Water deprivation and antidiuretic hormone (ADH) response testing can be useful if the patient is not azotemic. Elimination of other causes also allows a reasonable tentative diagnosis.

> NOTE: Renal failure typically produces urine specific gravities between 1.008 and 1.020, but rare dogs in renal failure may be hyposthenuric (1.006 to 1.007), and some dogs with 67% reduction in renal function experience concentration of urine to 1.027. Some cats may have a urine specific gravity greater than or equal to 1.035 with renal failure.

Persistent hyposthenuria suggests diabetes insipidus, although hyperadrenocorticism, hepatic insufficiency, and psychogenic polydipsia are also possible. Adrenal and hepatic function testing, water deprivation and ADH response testing, or both may be indicated. Water deprivation testing is unwise unless CBC, urinalysis, and biochemical profile have been evaluated first. Other medical causes of pu-pd are more common, and animals with such causes can be harmed by iatrogenic dehydration.

Dysuria

Alterations in behavior associated with urination (other than polyuria) generally suggest disorders affecting the urinary bladder, urethra, or both (Figure 7-2). Irritative or inflammatory (septic or nonseptic) lesions that do not impede urine flow typically cause animals to urinate small volumes and to

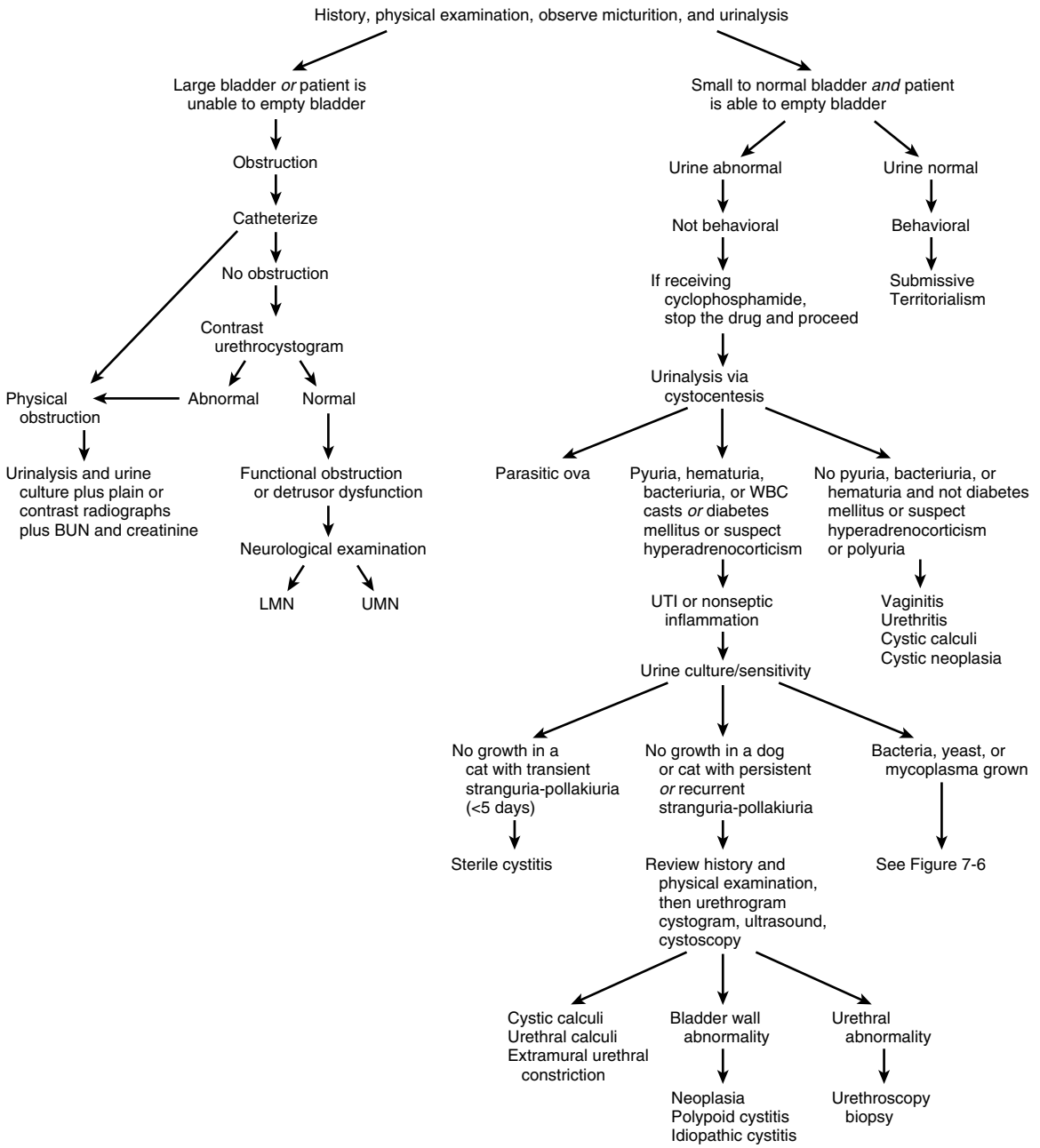

FIGURE 7-2. Diagnostic approach to dysuria in dogs and cats. BUN = blood urea nitrogen; LMN = lower motor neuron; UMN = upper motor neuron; UTI = urinary tract infection; WBC = white blood cell.

urinate more often, perhaps with apparent discomfort (i.e., dysuria). Urethral obstruction causes animals to make repeated voiding efforts that are either unproductive (i.e., complete obstruction) or somewhat productive but the bladder cannot be emptied (i.e., partial obstruction). Some animals with urethral obstruction have urinary incontinence. Such paradoxical incontinence occurs when accumulated urine is forced past an obstruction by markedly increased intravesicular pressure.

Urinary obstruction must be promptly identified, because uremia, hyperkalemia, and death occur within 48 to 72 hours of complete urethral obstruction, and severe structural damage occurs with chronic,

partial urethral, or ureteral obstruction. Urethral or trigonal obstruction typically causes an enlarged, turgid, and inexpressible bladder. Partial obstruction is more difficult to identify; however, observation of voiding plus assessment of residual urine volume in the bladder after micturition (i.e., palpation, ultrasonography, catheterization) is usually diagnostic. Palpating the urethra in male and female dogs and the prostate gland in male dogs is important. Mechanical obstructions, which may be intraluminal (e.g., uroliths, urethral plugs, neoplasms) or extraluminal (e.g., caused by displacement as the result of bladder entrapment in a perineal hernia, strictures, inflammation or edema of the urethra, severe prostatic diseases), are the most common. In these cases, passing a urinary catheter can be both diagnostic and therapeutic.

Even if a urinary catheter passes easily, a urethrocystogram with bladder distention should be performed to eliminate anatomic obstruction (a urinary catheter can pass by an obstruction, depending on the size of the catheter, the size of the urethra, and the nature of the obstruction). Detrusor muscle dysfunction and functional urethral obstruction (i.e., detrusor-sphincter dyssynergia) are neuromuscular causes of inability to urinate. Detrusor muscle dysfunction is usually secondary to prolonged bladder overdistention secondary to urethral obstruction or neurologic diseases. Detrusor-sphincter dyssynergia is diagnosed only when anatomic causes of obstruction have been excluded. With functional obstruction, neurologic examination usually detects deficits; however, these deficits may be subtle.

Most dysuric dogs that are not experiencing obstruction have lower urinary tract inflammation, UTI being a common cause. Finding bacteriuria, often with hematuria, proteinuria, and pyuria, identifies UTI. Urine culture is the definitive test (see Bacteriuria). If no evidence of UTI exists or if UTI does not respond as expected to therapy, calculi, neoplasia, cyclophosphamide therapy, and rarely parasitism of the bladder should be considered.

Cats with dysuria and inappropriate urination may have UTI, especially if they are over 10 years of age. Younger dysuric cats (i.e., 2 to 6 years of age), however, usually have idiopathic sterile cystitis (i.e., feline urologic syndrome, feline interstitial cystitis, feline idiopathic cystitis). This is a diagnosis of exclusion; urinalysis is needed. Cats with sterile cystitis usually have hematuria with little or no pyuria. The second most common cause of these signs is urolithiasis. Idiopathic sterile cystitis typically resolves spontaneously within 7 days. If the problem does not resolve, radiography or ultrasonography of the urinary bladder or both may be needed.

If surgery is performed because of a lower urinary tract problem, the bladder and, in male dogs, the prostate gland should undergo biopsy regardless of how normal or neoplastic the organ appears. Bladder abnormalities may occur without gross lesions, whereas polypoid cystitis may mimic malignancy.

URINALYSIS

Commonly Indicated • Urinalysis is part of a thorough evaluation of patient health, especially in ill animals. It is essential in patients with a urinary tract problem.

Advantages • Urinalysis is easy and inexpensive to perform and provides important information in patients with various systemic diseases.

Analysis • Urine is preferably obtained by cystocentesis. (The exception is patients with known coagulopathy or suspected mild hematuria in which a voided sample is preferred to avoid mild iatrogenic hemorrhage associated with cystocentesis.) If cystocentesis cannot be performed, a midstream voided or catheterized sample can be used. The method of collection should be recorded so abnormalities can be correctly interpreted.

For greatest accuracy (especially regarding casts, cellular components, and crystalluria), urine sediment should be examined while the specimen is as fresh as possible. If the urinalysis cannot be performed within 30 minutes, the urine should be held at 4°C and brought back to room temperature just before analysis.

Analysis consists of four important steps: (1) determining color and turbidity, (2) chemical analysis using multitest dipsticks, (3) measurement of specific gravity, and (4) microscopic analysis of the sediment. The clinician should thoroughly mix the sample and determine the color and turbidity first; then immerse the reagent strip into the urine and rapidly remove it, tapping the edge of the strip on the edge of the container to remove excess urine. The dipstick should be held level to avoid runoff between pads and prevent

mixing reagents from different test pads. The clinician should compare the color of the pads with the color scale provided by the manufacturer at the proper time interval and determine urine specific gravity using a refractometer. The accuracy of the refractometer should be checked periodically to be sure that a reading of 1.000 is obtained with distilled water.

Next, 3 to 5 ml of urine should be centrifuged at 1500 to 2000 rpm for 5 minutes. Standardization of volume, speed, and duration of centrifugation is important for comparing results from different samples. If the uncentrifuged urine sample was visibly hemorrhagic or very turbid, the clinician should repeat the dipstick analysis and the specific gravity on the supernatant. Next, most of the supernatant should be decanted, leaving approximately 0.5 ml in the tube, and the sediment in the remaining supernatant should be resuspended. Next, the clinician should transfer a drop of the reconstituted sediment to a microscope slide and place a coverslip over it. The intensity of the microscope light should be dimmed, and the clinician should examine under low power (10x) for casts, crystals, and cells. The number of casts per low-power field (lpf) should be counted, and then the specimen should be examined under high power (40x) to identify cells and bacteria. The clinician should count the number of WBCs and RBCs per high-power field (hpf) and estimate the number of bacteria (i.e., trace, moderate, many). Finally, the results should be recorded, with a notation of how urine was collected and how many milliliters of urine were spun if it was less than 3 to 5 ml.

Color and Turbidity

Analysis • Color and turbidity are analyzed by visual inspection. Normal urine is clear to slightly turbid and light yellow to amber. Dilute urine tends to be colorless, and concentrated urine is a darker yellow. Different colors and their significance are listed in Table 7-2. Significant disease may exist even if the urine is normal in color and turbidity. If urine discoloration is noted, the clinician should review the patient's history for drug administration and carefully examine the urine sediment. Hematuria, hemoglobinuria, and bilirubinuria are the most common causes of discolored urine. Pyuria, hematuria, crystalluria, and lipiduria are common causes of increased turbidity.

Specific Gravity

Analysis • Specific gravity is determined with a refractometer. The clinician should periodically check the refractometer's calibration by verifying that distilled water gives a reading of 1.000. Some dipsticks have a test pad that indicates specific gravity; however, these results are often inaccurate, especially when the specific gravity is greater than 1.025. Use of dipstick test pads to evaluate urine specific gravity is not recommended.

Normal Values • Specific gravity varies in normal dogs and cats, and any random specific gravity may be normal in euhydrated animals. Specific gravities less than 1.020 may be associated with evident polyuria. Specific gravity is important to assess renal function in dehydrated or azotemic animals. The urine

TABLE 7-2. Potential Causes of Discolored Urine in Dogs and Cats

URINE COLOR	CAUSES	URINE COLOR	CAUSES
Dark yellow	Concentrated urine	Yellow-brown	Bile pigments
Pale yellow	Normal urochromes, urobilin	Red	Hemoglobin
			RBCs
			Myoglobin
Yellow-orange	Bilirubin		Dyes
	Fluorescein		Phenazopyridine
	Concentrated urine		Phenolsulfonphthalein
	Phenazopyridine		
Green-blue	Methylene blue	Milky	Pyuria
	Dithiazanine		Lipiduria
	Biliverdin		Phosphate crystals
Brown-black	Bile pigments	Colorless	Dilute
	Myoglobin		
	Methemoglobin		

RBCs = red blood cells.

of a dog with evident dehydration and normal renal function should have a specific gravity greater than 1.030, and a cat's should be greater than 1.035.

Danger Values • None.

Artifacts: Drug Therapy That May Alter Results • Low urine specific gravity may be caused by glucocorticoids, diuretics, anticonvulsants, excessive thyroid hormone supplementation, persistent use of very-low–protein or high-salt diets, methoxyflurane, aminoglycosides, and fluid therapy. Increased specific gravity may be caused by radiographic contrast media if the preadministration urine specific gravity was less than 1.040 (if preadministration urine specific gravity was >1.040, urine specific gravity may decrease because of osmotic diuresis).

NOTE: It is important to obtain urine specific gravity before treatment, especially fluid or diuretic therapy.

Causes of Altered Urine Specific Gravity • Urine that is less than or equal to 1.007 is hyposthenuric. Hyposthenuria indicates renal function capable of diluting glomerular filtrate and suggests that renal failure is absent; however, some dogs with renal failure excrete mildly hyposthenuric urine. Persistent hyposthenuria suggests a lack of ADH (central diabetes insipidus), excessive water consumption (primary polydipsia), resistance to ADH (nephrogenic diabetes insipidus), or loss of medullary tonicity. Primary polydipsia can be caused by hyperthyroidism, hypercalcemia, hypokalemia, or hepatic failure, or it can be "psychogenic." Resistance to ADH may be caused by secondary nephrogenic diabetes insipidus (e.g., hyperadrenocorticism, hypercalcemia, hypokalemia, pyelonephritis, pyometra, *Escherichia coli* septicemia, hypoadrenocorticism). Primary nephrogenic diabetes insipidus is rare and is due to congenital lack of ADH receptor responsiveness. An increased solute load (e.g., glucosuria, posturethral obstruction, increased salt intake) can also cause polyuria, as can decreased medullary tonicity (e.g., hepatic failure, a very-low–protein diet, hyponatremia, chronic diuretic therapy).

Urine with a specific gravity of 1.008 to 1.012 is isosthenuric, meaning that the kidneys have not altered the concentration of the glomerular filtrate. Urine with a specific gravity of 1.013 to 1.029 (dog) or 1.013 to 1.034 (cat) has been concentrated, but not enough to document adequacy of renal tubular function.

Urine with a specific gravity of greater than 1.030 (dog) demonstrates a concentrating ability sufficient to indicate adequate renal function to maintain normal homeostasis. A patient with a specific gravity of urine greater than 1.030 could still have many of the diseases that cause pu-pd (e.g., hyperadrenocorticism, hepatic insufficiency, hyperthyroidism), as well as renal glomerular disease.

A single urine specific gravity greater than 1.007 and less than 1.030 (dog) or 1.035 (cat) does not imply renal tubular dysfunction or pu-pd unless the patient is clinically dehydrated or azotemic, in which case such a specific gravity reflects abnormal renal tubular function. Otherwise, one must document failure to concentrate urine adequately during water deprivation testing to establish urine concentrating ability as abnormal. Persistently hyposthenuric or isosthenuric urine is an indication for further testing (see Figure 7-1).

Urine pH

Analysis • Urine pH is measured with a pH test pad on a urine reagent strip. Meters to measure pH are more accurate but are expensive.

Normal Values • Normal dogs and cats may have a urine pH of 5.0 to 8.5.

Danger Values • None.

Artifacts • Falsely increased: standing open at room temperature, which leads to loss of CO_2; contamination by detergents or disinfectants.

Drug Therapy That May Alter Results • Decreased urine pH may be the result of urinary acidifiers such as methionine, mandelate, phosphate salts, and ammonium chloride. Increased urine pH may be caused by acetazolamide, bicarbonate, and potassium citrate.

Causes of Acid or Alkaline Urine • Any urine pH can be normal. Urine pH is a crude index of acid-base balance and is not a reliable index of blood pH (e.g., a vomiting patient with secondary hypochloremia may have aciduria despite systemic alkalosis, because it is conserving bicarbonate as an anion). Causes of acid urine include ingestion of meat, respiratory and metabolic acidosis,

severe vomiting with chloride depletion, severe diarrhea, starvation, pyrexia, and administration of urinary acidifiers. Causes of alkaline urine include a recent meal (i.e., postprandial alkaline tide), ingestion of alkali (e.g., bicarbonate or citrate), UTI with urease-producing bacteria (typically *Staphylococcus* or *Proteus* spp.), renal tubular acidosis (RTA), diets rich in vegetables and cereals, and metabolic and respiratory alkalosis.

Persistently alkaline urine is an indication for complete urinalysis and urine culture. If no reason for alkaline urine is found on history, urinalysis, or urine culture, distal RTA may be considered, although it is rare. Both distal and proximal RTA cause hyperchloremic metabolic acidosis with a normal anion gap and often produce hypokalemia (see Chapter 6).

Proteinuria

Analysis • Urine protein is usually measured with qualitative tests such as a urine reagent strip (i.e., dipstick) or by precipitation (i.e., sulfosalicylic acid, nitric acid). The dipstick is more sensitive to albumin than to globulins. Spectrophotometric analysis is more precise; it is discussed under urine protein:urine creatinine ratio.

Proteinuria must be interpreted in light of urine specific gravity. Because screening tests are qualitative, more protein must be lost into diluted urine than into concentrated urine to give the same result (e.g., a trace reaction with a specific gravity of 1.010 means that more protein is being lost into the urine than with the same trace reaction with a 1.030 specific gravity).

Myelomas producing free light chains (i.e., Bence Jones protein) do not cause a positive dipstick reaction but are positive on precipitation testing. Protein electrophoresis is indicated in such patients, in which case most of the urinary protein is a monoclonal spike in the β or γ regions.

Normal Values • A trace or 1+ reaction is considered normal with a specific gravity greater than 1.035. Any protein is potentially abnormal with a specific gravity less than 1.035. These values are approximations. The tests are so sensitive that the amount of protein being lost in urine with a 4+ reaction may vary widely between animals. More quantitative tests are needed to precisely determine severity of protein loss (see Urine Protein:Urine Creatinine Ratio).

Danger Values • None.

Artifacts. • Falsely decreased: (sulfosalicylic acid) very alkaline urine; (dipstick) presence of Bence Jones proteins. Falsely increased: (sulfosalicylic acid) radiographic contrast media; (dipstick) phenazopyridine, chlorhexidine, allowing the test pad to become wet during storage, allowing prolonged contact of the test pad with excessive amounts of urine, or highly alkaline urine [dipstick, pH ≥ 9]).

Drug Therapy That May Cause Proteinuria • Any drug that causes renal tubular or glomerular injury can cause proteinuria (Table 7-3).

Causes of Proteinuria • One must first decide if the proteinuria is significant or not by examining the specific gravity, as described earlier (Figure 7-3). If it is insignificant, one may ignore it unless the patient is receiving nephrotoxic drugs (e.g., aminoglycosides). Such drugs should be stopped regardless of the amount of proteinuria, because mild proteinuria may be an early sign of nephrotoxicity and impending acute renal failure. Aminoglycoside nephrotoxicity typically causes proteinuria or other urinalysis changes (e.g., isosthenuria, glucosuria, cylindruria) before azotemia.

TABLE 7-3. Selected Potentially Nephrotoxic Drugs*

Aminoglycoside antibiotics such as neomycin, kanamycin, gentamicin, amikacin, and tobramycin *(important)*
Amphotericin B *(important)*
Arsenic
Cephalothin *(uncommon)*
Cisplatin *(important)*
Cyclophosphamide (nephrotoxicity is uncommon; sterile cystitis is more common)
Dextran (low molecular weight)
Ethylene glycol *(important)*
Furosemide *(uncommon)*
Heavy metals (i.e., gold, lead, mercury)
Nonsteroidal anti-inflammatory drugs such as aspirin, ibuprofen *(important* when there is preexisting renal disease or hypotension*)*
Polymyxin B *(important)*
Radiographic contrast media *(important* when there is preexisting azotemia and dehydration*)*
Sulfonamides *(uncommon* if more soluble sulfonamides are used*)*
Tetracyclines *(uncommon)*
Thallium
Thiazides *(uncommon)*
Vancomycin *(uncommon)*
Zinc

*Not all these drugs reliably produce nephrotoxicity. Those drugs recognized as the most dangerous are denoted *important*.

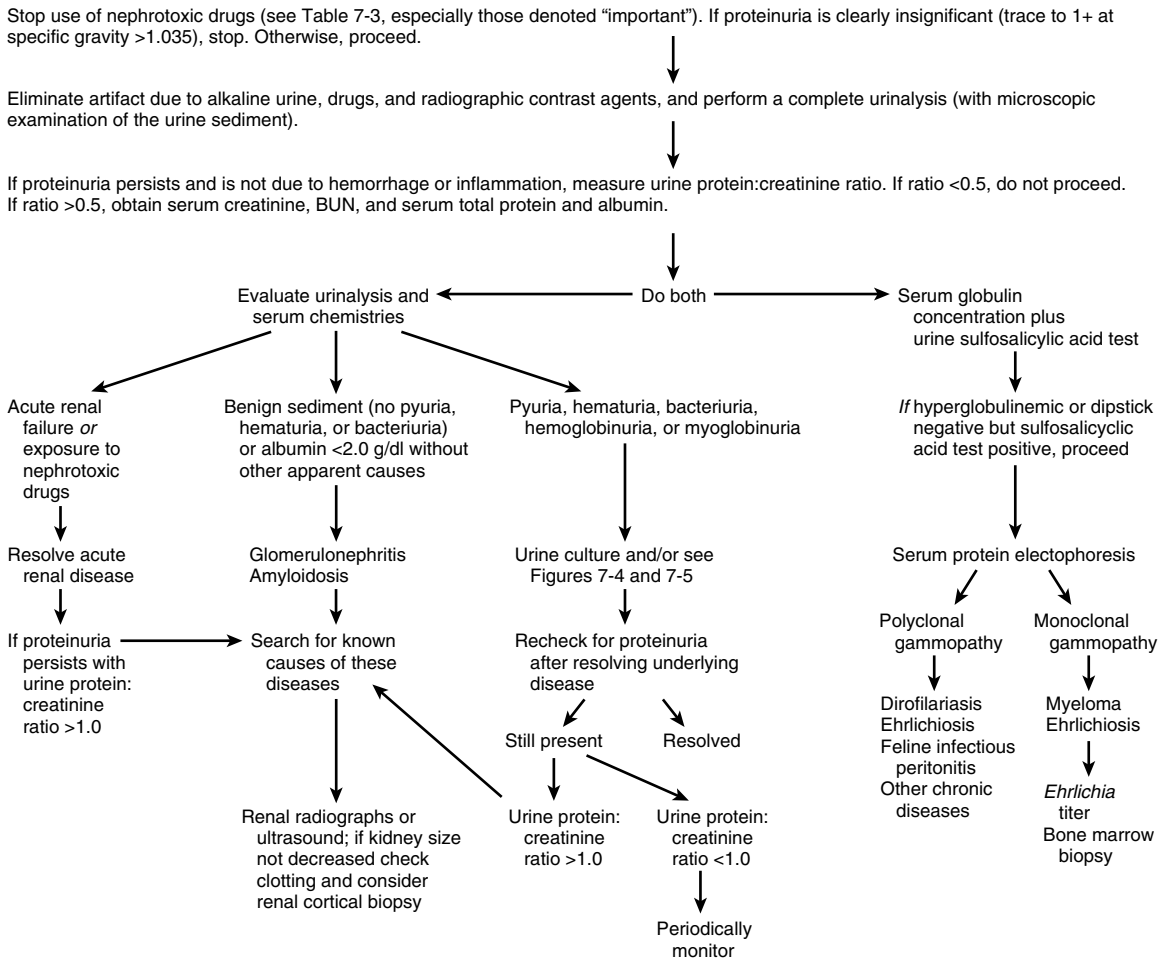

FIGURE 7-3. Diagnostic approach to proteinuria in dogs and cats. BUN = blood urea nitrogen.

If the proteinuria is abnormal, urinalysis with sediment examination is performed to exclude hemorrhage and inflammation as the cause. Proteinuria associated with inflammation (i.e., pyuria) or hemorrhage (i.e., hematuria) requires resolution of the inflammation or hemorrhage and then rechecking for persistence of proteinuria. The most common cause of inflammatory proteinuria is UTI. Urine culture should be performed if infection is possible.

One should repeat the urinalysis to determine if proteinuria is persistent if hemorrhage and inflammation are excluded by a normal ("quiet" or "inactive") urine sediment. Transient (i.e., functional) proteinuria has many causes (e.g., strenuous exercise, fever, seizures, venous congestion of the kidneys) and is rarely significant. Persistent proteinuria with an inactive urine sediment should prompt determination of a urine protein:urine creatinine ratio to ascertain its severity.

If proteinuria is abnormal, evaluation of serum albumin and globulin concentrations is indicated (see Figure 7-3).

NOTE: A normal serum albumin concentration does not mean that proteinuria is insignificant. Mild proteinuria (i.e., urine protein:urine creatinine ratios of 0.6 to 3) may be associated with renal tubular or glomerular diseases.

Marked proteinuria (i.e., urine protein:urine creatinine ratio > 3) associated with a quiet sediment and normal serum globulins or a polyclonal gammopathy is usually the result of renal glomerular disease (i.e., glomerulonephritis, amyloidosis). (NOTE: Feline amyloidosis usually affects the renal medulla

and may not cause proteinuria.) One should search for causes of glomerulonephropathy (e.g., chronic parasitic disease such as heartworm disease, hepatozoonosis; immune diseases such as systemic lupus erythematosus; chronic infectious diseases such as borreliosis, feline leukemia virus [FeLV] infection, feline immunodeficiency virus [FIV] infection, ehrlichiosis; other chronic inflammatory diseases; neoplasia; hyperadrenocorticism). If no underlying disease is identified or if proteinuria persists despite treatment for an identified disease, renal cortical biopsy may be performed to determine whether glomerulonephritis or amyloidosis is present.

Proteinuria detected by precipitation testing but not dipstick, or proteinuria associated with a monoclonal gammopathy may be caused by Bence Jones proteins. This necessitates a search for osteolytic or lymphoproliferative lesions. Ehrlichiosis occasionally mimics myeloma (i.e., glomerulonephritis, monoclonal-like gammopathy, bone marrow plasmacytosis). A titer may be diagnostic (see Chapter 15). If such a patient does not have ehrlichiosis, urine protein electrophoresis is indicated. A monoclonal spike in the urine and serum strongly suggests myeloma (see Chapter 12).

Urine Protein:Urine Creatinine Ratio

Indication • Measurement of the urine protein:urine creatinine ratio is indicated to determine approximate magnitude and therefore the significance of proteinuria.

Advantages • Measurement of the urine protein:urine creatinine concentrations is more accurate than dipstick procedures, requires only a single random urine sample, and correlates well with 24-hour determinations.

Disadvantages • A complete urinalysis should be performed on an aliquot of the same sample to look for hemorrhage or inflammation, which alters assessment of the results. Day-to-day variation in animals with glomerular disease has not been studied, so the ratio is not useful to monitor disease progression. This ratio gives no information about the origin of the proteinuria, it only quantifies it.

Analysis • Urine protein and creatinine concentrations are measured spectrophotometrically. Total protein is usually determined by Coomassie brilliant blue dye-binding or trichloroacetic acid–ponceau S methods. Measurements with the former tend to be higher than with the latter. The following ratio is calculated:
Total protein (mg/dl)/creatinine (mg/dl)
Ratios greater than 1.0 are abnormal.

Normal Values • Dogs, less than 0.3; cats, less than 0.6.

Danger Values • None.

Artifacts • See discussions of total protein (Chapter 12) and creatinine (this chapter).

Causes of Increased Urine Protein: Urine Creatinine Ratio • See the earlier discussion under Proteinuria.

Glucosuria

Analysis • Urine glucose concentration is measured using a test pad on urine dipsticks or paper test strip (i.e., glucose oxidase method) or with a test for reducing substances in urine (Clinitest).

Normal Values • Dog and cat urine should be negative for glucose by these tests.

Danger Values • None.

Artifacts • Falsely decreased: refrigerated urine, large amounts of ascorbic acid, tetracycline (caused by ascorbic acid in formulation), plus low urine pH and increased urine salt concentrations (paper test strip). Falsely increased: hydrogen peroxide, hypochlorite or bleach, and a nonglucose oxidizing substance in the urine of cats with urethral obstruction (dipstick).

The Clinitest reaction is not specific for glucose. Falsely increased: galactose, pentose, lactose, fructose, salicylates, penicillins, some cephalosporins, radiographic contrast media, and large amounts of ascorbic acid or sulfonamides. Very strong positive reactions may be read out low because the final color is less orange than that which occurred during the reaction (i.e., "pass-through" phenomenon).

Drug Therapy That May Cause Glucosuria • Glucosuria may be caused by drugs capable of producing hyperglycemia (see Chapter 8), intravenous (IV) infusion of dextrose-containing solutions, and selected nephrotoxins causing proximal renal tubular dysfunction (e.g., aminoglycoside nephrotoxicity).

TABLE 7-4. Causes of Glucosuria in Dogs and Cats

Blood glucose concentration exceeding renal threshold	Diabetes mellitus Stress (especially in cats) Infusion of dextrose-containing fluids Hyperadrenocorticism (rarely causes glucose >180 mg/dl) Pheochromocytoma (rare)
Abnormal proximal renal tubular function	Aminoglycoside toxicity Acute renal failure Fanconi's syndrome Primary renal glucosuria
Contamination	Urinary hemorrhage in a patient with mild hyperglycemia

Causes of Glucosuria • Glucosuria usually occurs because the renal threshold for glucose reabsorption is exceeded because of hyperglycemia (i.e., blood glucose >180 mg/dl in dogs and >300 mg/dl in cats). Glucosuria always necessitates measuring blood glucose. The most common cause of glucosuria because of hyperglycemia is diabetes mellitus (Table 7-4). If blood glucose concentration is normal, urine should be reevaluated with both urine dipstick and the Clinitest. If glucosuria is still present, proximal renal tubular dysfunction is likely. History should be reviewed for nephrotoxins and urinalysis, and blood urea nitrogen (BUN) and serum creatinine concentrations measured to search for proximal renal tubular disease. Occasionally, multiple tubular defects are found: Fanconi's syndrome occurs in some breeds (e.g., Basenji), producing glucosuria despite normoglycemia, hyperchloremic metabolic acidosis, hyperphosphaturia, and aminoaciduria.

Ketonuria

Analysis • A test pad on urine dipstick or tablet (Acetest) is commonly used to measure ketone concentration. These detect acetoacetate and acetone but not beta-hydroxybutyrate (which is responsible for acidosis).

Normal Values • Urine results should be negative for ketones.

Danger Values • Severity of ketoacidosis is not necessarily correlated with the degree of ketonuria. Large amounts of urine ketones plus lethargy and vomiting strongly suggest ketoacidosis and warrant immediate measurement of blood glucose plus evaluation of acid-base status (e.g., total carbon dioxide [Tco_2] determination, blood gas analysis).

Artifacts • Falsely increased: phenazopyridine, dimercaprol, aspirin, captopril, mesna, n-acetylcysteine, and valproic acid.

Drug Therapy That May Cause Ketonuria • Streptozotocin and aspirin intoxication.

Causes of Ketonuria • Lipolysis produces ketones. Starvation, fasting, and diabetic ketoacidosis are the most common causes. If ketonuria and glucosuria are present, diabetes mellitus is highly likely and should be confirmed by measurement of blood glucose. If a patient is ketonuric, glucosuric, and hyperglycemic, diabetes mellitus is diagnosed and serum sodium, potassium, phosphorus, and Tco_2 or blood gas determinations are indicated. Ketonuria without glucosuria suggests excessive lipid catabolism and is not generally investigated further in anorexic, nondiabetic patients.

Bilirubinuria

Analysis • A test pad on urine dipsticks (i.e., diazo method) and occasionally the oxidation method (i.e., Harrison's spot test) are used to test for bilirubinuria. The tablet method may be more sensitive than the dipstick.

Normal Values • Dogs (especially males) may have small amounts of bilirubinuria if the urine specific gravity is greater than or equal to 1.030. Normal cats do not have bilirubinuria.

Danger Values • None.

Artifacts • Falsely decreased: prolonged exposure to ultraviolet light or standing at room temperature exposed to air (bilirubin oxidizes into biliverdin, which is not detected). Substantial hemoglobinuria also may cause a falsely decreased reading by masking the bilirubin-induced color change on dipsticks. Falsely increased: large amounts of phenothiazines.

Drug Therapy That May Cause Bilirubinuria • See Chapters 3 and 9 for causes of hemolytic anemia and icterus, respectively.

Causes of Bilirubinuria • Bilirubin must be conjugated to be excreted into urine. The liver is principally responsible for conjugation,

but canine kidneys can also conjugate bilirubin. The most common causes of hyperbilirubinemia in dogs and cats are hepatic disease, posthepatic bile duct obstruction, and hemolytic diseases. Mild bilirubinuria may result from prolonged anorexia.

Excessive bilirubinuria in a dog or any bilirubinuria in a cat is an indication to determine total serum bilirubin concentration, SAP and ALT activities, and a hematocrit. If the hematocrit is below the lower limit of normal, a CBC plus reticulocyte count is indicated. See Chapter 9 for the diagnostic approach to icterus.

Urobilinogen

Assay • A test pad on urine dipsticks are used to test for urobilinogen.

Normal Values • 0.1 to 1.0 Ehrlich units. One cannot detect total absence of urobilinogen with this test.

Danger Values • None.

NOTE: This is a poor test, the results of which should be ignored.

Occult Blood

Analysis • Test pad for occult blood on most urine dipsticks detects hemoglobin, myoglobin, and, to a lesser extent, intact red blood cells (RBCs). The assay is very sensitive, detecting 0.03 mg hemoglobin/dl.

Normal Values • No hemoglobinuria or myoglobinuria. A few RBCs (i.e., five or fewer/hpf) may occur in normal urine. Higher numbers of RBCs are found in voided urine in proestral bitches.

Danger Values • None, although marked hemoglobinuria or myoglobinuria in a dehydrated animal may cause renal tubular injury.

Artifacts • Falsely decreased: ascorbic acid and captopril. Falsely increased: flea dirt in sample.

Causes of Positive Occult Blood • Hematuria is the most common cause of a positive dipstick finding of occult blood; therefore, a urine sediment examination should be performed. If RBCs or RBC ghosts are found in the sediment, hematuria is confirmed. Diluted or alkaline urine may cause RBC lysis, and hemolyzed RBCs are not always visible. Hemoglobinuria because of hematuria is followed up as for hematuria (Figure 7-4).

If no RBCs are found, especially if the urine is grossly discolored after centrifugation, one should determine hematocrit and plasma color. If the plasma is pink or red despite proper venipuncture technique (hemoglobinemia) (Figure 7-5), hemoglobinuria is present. Hemoglobinuria because of hemoglobinemia indicates hemolytic anemia (see Chapter 3), and a complete CBC is indicated. If no evidence of hematuria or hemolysis exists, myoglobinuria must be considered. One may test for myoglobinuria by requesting urine precipitation with 80% saturated ammonium sulfate. If the urine supernate remains red-brown after centrifugation, 2.8 g ammonium sulfate should be added to 5 ml of urine with a neutral pH. After centrifuging this mixture, if the supernate remains dark, myoglobin is confirmed. Occasional interpretation problems occur if the urine is colored because of nonprotein pigments. Myoglobinuria requires a search for rhabdomyolysis or myositis (see Chapter 14), and serum creatine kinase activity should be measured.

If no evidence of hematuria, hemoglobinemia, hemolysis, or muscle disease is seen, one should recheck for artifacts. Persistent hemoglobinuria of unknown cause necessitates looking for occult urinary hemorrhage.

Hematuria

Analysis • A test pad on a urine dipstick for occult blood or microscopic examination of urine sediment is used to test for hematuria.

Normal Values • Less than five RBCs/hpf. If urine is obtained by cystocentesis or catheterization, iatrogenic trauma in obtaining the sample can cause gross or microscopic hematuria.

Danger Values • None.

Artifacts • Falsely decreased: hemolysis occurs rapidly in hyposthenuric or alkaline urine and may be complete within 2 hours. This causes a positive occult blood reaction with no obvious RBCs in the urine sediment, although RBC ghosts are sometimes visible in the sediment.

Causes of Hematuria • The clinician should first consider iatrogenic hemorrhage

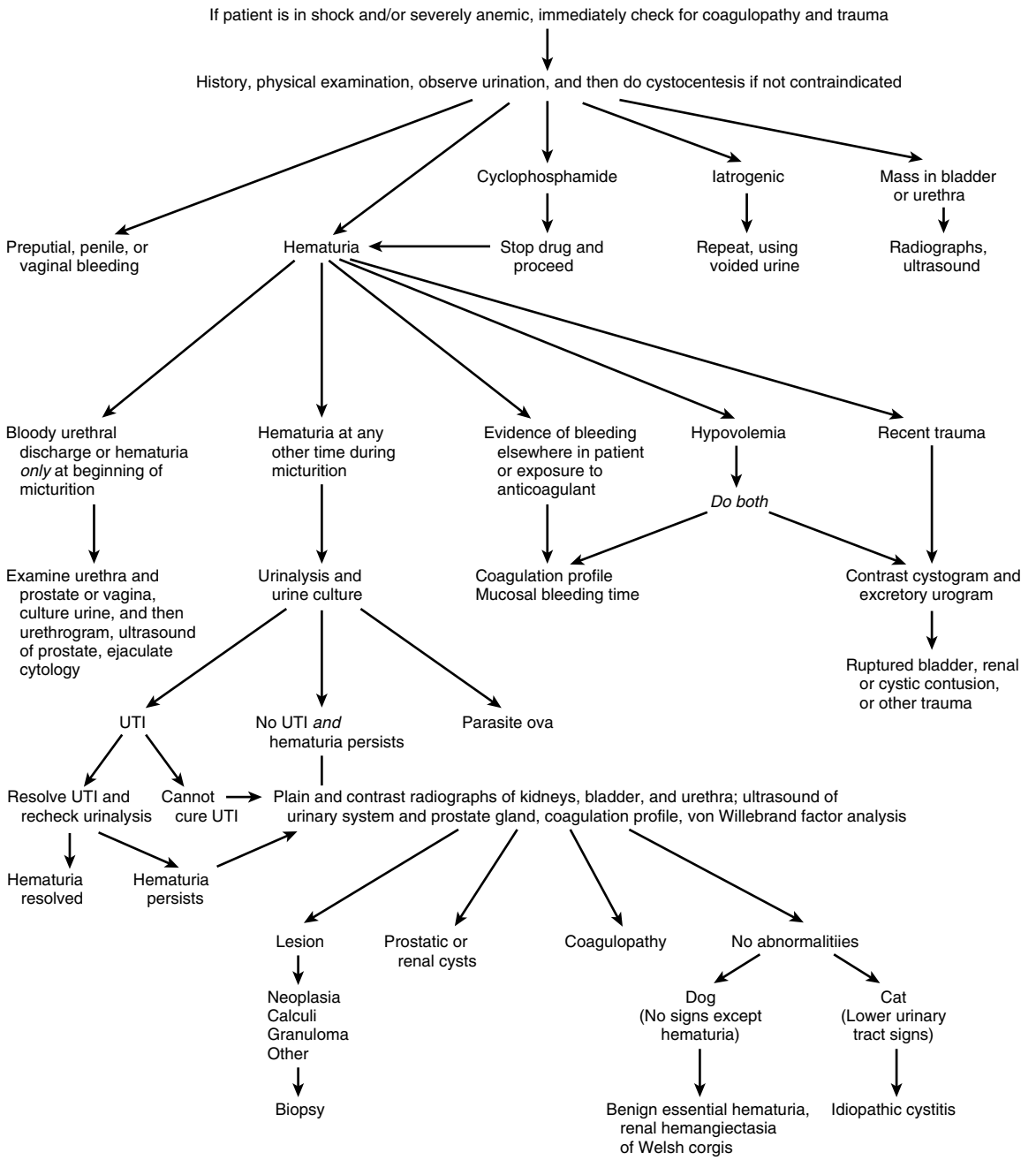

FIGURE 7-4. Diagnostic approach to persistent hematuria (gross or microscopic) in dogs and cats. UTI = urinary tract infection.

during sampling (see Figure 7-4). Using voided urine avoids the possibility of iatrogenic hemorrhage during catheterization or cystocentesis. If gross hematuria is present, timing of the most intense urine discoloration during the urine stream helps localize the bleeding site. Blood independent of urination or most severe at the beginning of urination suggests the urethra, prostate gland, or prepuce in male dogs or the uterus or vagina of females. Blood at the end of urination suggests the bladder as the site of origin. Bleeding at any site can cause blood throughout urination. If urine collected by

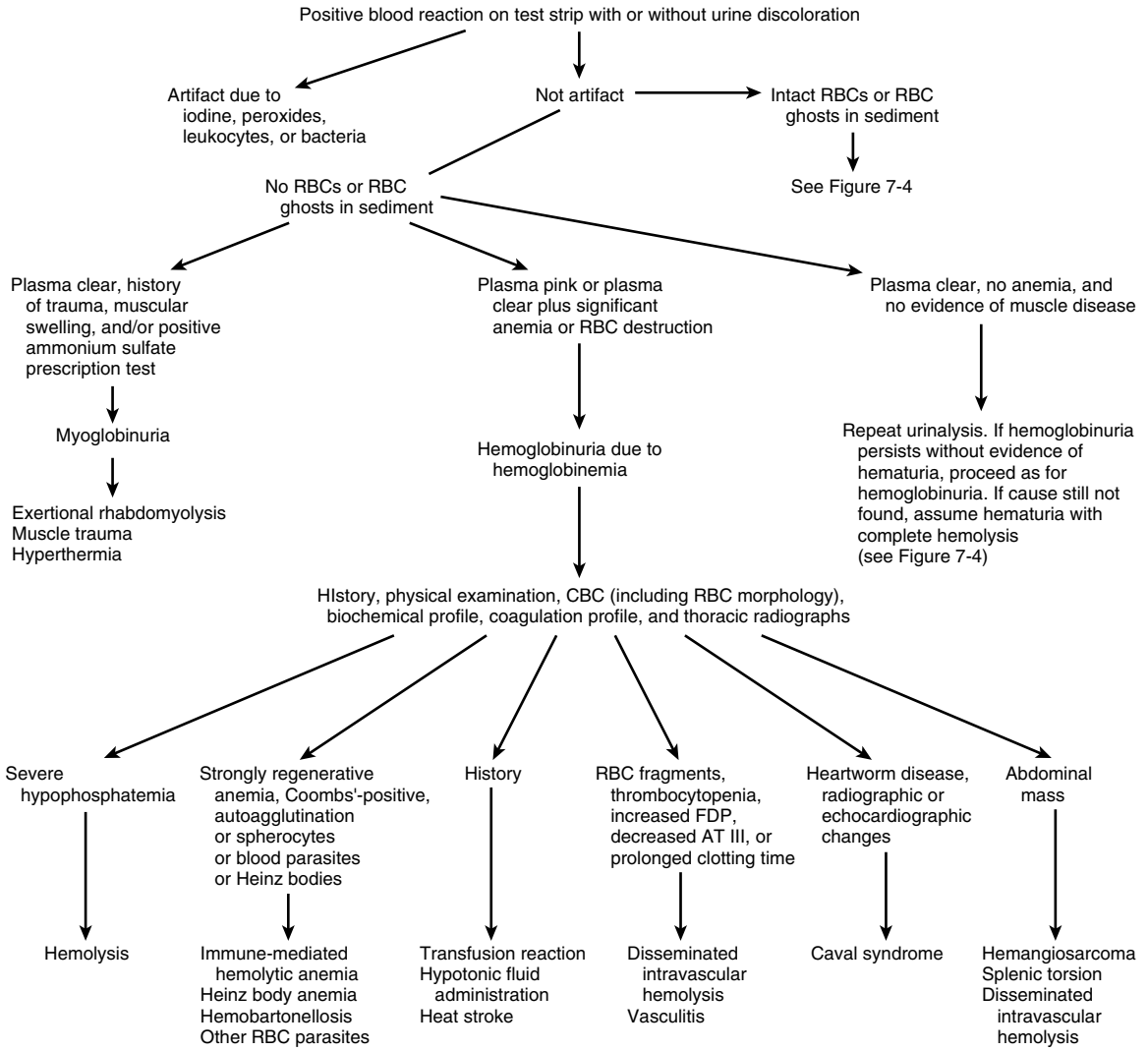

FIGURE 7-5. Diagnostic approach to hemoglobinuria in dogs and cats. AT III = antithrombin III; CBC = complete blood count; FDP = fibrin degradation products; RBC = red blood cell.

cystocentesis has no blood but voided urine has blood, the distal urethra, prepuce, vagina, or uterus is most likely. Blood from prostatic and proximal urethral lesions usually refluxes back into the bladder as well as causing bleeding independent of urination.

Hematuria may result from infection, calculi, other nonseptic inflammation, coagulopathies, trauma (i.e., exogenous, iatrogenic), neoplasia, cysts, renal infarcts, chronic passive renal congestion, urinary parasites, strenuous exercise, estrus in females, or glomerulonephritis (rare).

In dogs, UTI is the most common cause of hematuria; therefore, a urine culture is indicated even if pyuria and bacteriuria are absent. Calculi are another common cause, and survey abdominal radiographs are indicated. If the patient has a history of anticoagulant exposure, evidence of coagulopathy on history or physical examination, hypovolemia or anemia because of hemorrhage, or no obvious genitourinary cause of the hemorrhage, coagulation screening tests (see Chapter 5) are indicated. If all these tests are unrevealing, contrast radiography, ultrasonography, or both are used to examine kidneys, ureters, urinary bladder, prostate, and urethra. In male dogs, prostatic fluid (ejaculate or postprostatic massage) should be examined.

In cats, idiopathic cystitis (i.e., feline urologic syndrome, feline idiopathic cystitis, feline interstitial cystitis) is a common cause of hematuria. Prostatic disease is an uncommon cause of feline hematuria. Other than these, the causes for hematuria are similar in cats and dogs.

Nitrituria

Analysis • A test pad on urine dipsticks are used to measure for nitrituria.

Normal Values • Negative.

Danger Values • None.

NOTE: This test is inaccurate in dogs and cats and should be ignored.

Pyuria

Analysis • Microscopic examination of urine sediment. The leukocyte esterase test strip used to evaluate human urine for WBC is insensitive in dogs and unevaluated in cats. Results from the test strip should not be substituted for urine sediment examination.

Normal Values • Less than three WBCs/hpf. Samples obtained by cystocentesis are preferred to avoid distal urethral and reproductive tract contamination.

Danger Values • None.

Artifacts • Falsely decreased: alkaline urine, dilute urine, or prolonged exposure to room temperature causes WBC lysis.

Causes of Pyuria • Pyuria indicates inflammation. Preputial or vaginal secretions can also be responsible. A cystocentesis-obtained urine sample should be used to eliminate these sites. UTI is the most common cause of pyuria; calculi and neoplasia are other common causes. Urine culture is indicated. (NOTE: Diluted urine or urine from patients with impaired WBC function, such as hyperadrenocorticism or diabetes mellitus, may not have pyuria despite a UTI.) If pyuria persists and bacteria cannot be cultured, survey and contrast radiographs and/or ultrasonograph of the urinary tract are indicated to eliminate calculi and neoplasia.

Bacteriuria

Analysis • Microscopic examination of urine sediment or urine culture (see Chapter 15) is used to test for bacteriuria. Cystocentesis-obtained urine is preferred to avoid contamination from the distal urethra and reproductive tract. Midstream catheterized samples from male dogs and from cats of both sexes are acceptable, however. Catheterized samples from female dogs should be avoided because of the risk of significant contamination of sample from vaginal organisms and the risk of introducing infection.

Normal Values • Bacteriuria is abnormal in urine obtained by cystocentesis. Quantitative urine culture is needed to determine the significance of bacteria in urine obtained by catheterization. Bacteria in voided urine could be the result of infection or contamination with normal flora of distal urethra and genital tracts.

Danger Values • None.

Artifacts • Falsely decreased: recent antibiotic therapy, diuresis, contamination of urine with oxidants (e.g., bleach), or delay between collection and examination. Falsely increased: delay in examination with urine remaining at room temperature and contaminated centrifuge tubes or stain solutions. Brownian motion of amorphous debris may be confused with bacteriuria in unstained, wet mount preparations.

Causes of Bacteriuria • Once artifacts and contamination are eliminated, bacteriuria allows diagnosis of UTI (Figure 7-6). Bacteriuria, pyuria, and hematuria in properly obtained urine are classic findings for UTI; however, not all UTIs have detectable pyuria, hematuria, or bacteriuria. Dilute urine may have such a low concentration of cells they cannot be found in the sediment. Greater than 10^4 rods/ml or 10^5 cocci/ml must be present before they can readily be seen in the urine sediment. Patients with hyperadrenocorticism (endogenous or iatrogenic) or diabetes mellitus may not have any evidence of UTI on urinalysis. Therefore it is reasonable to culture urine from all patients with these conditions.

Urine culture is recommended in all patients with suspected UTI. If culture is not performed, bacterial morphology (rods or cocci) and urine pH (Table 7-5) can be used to predict

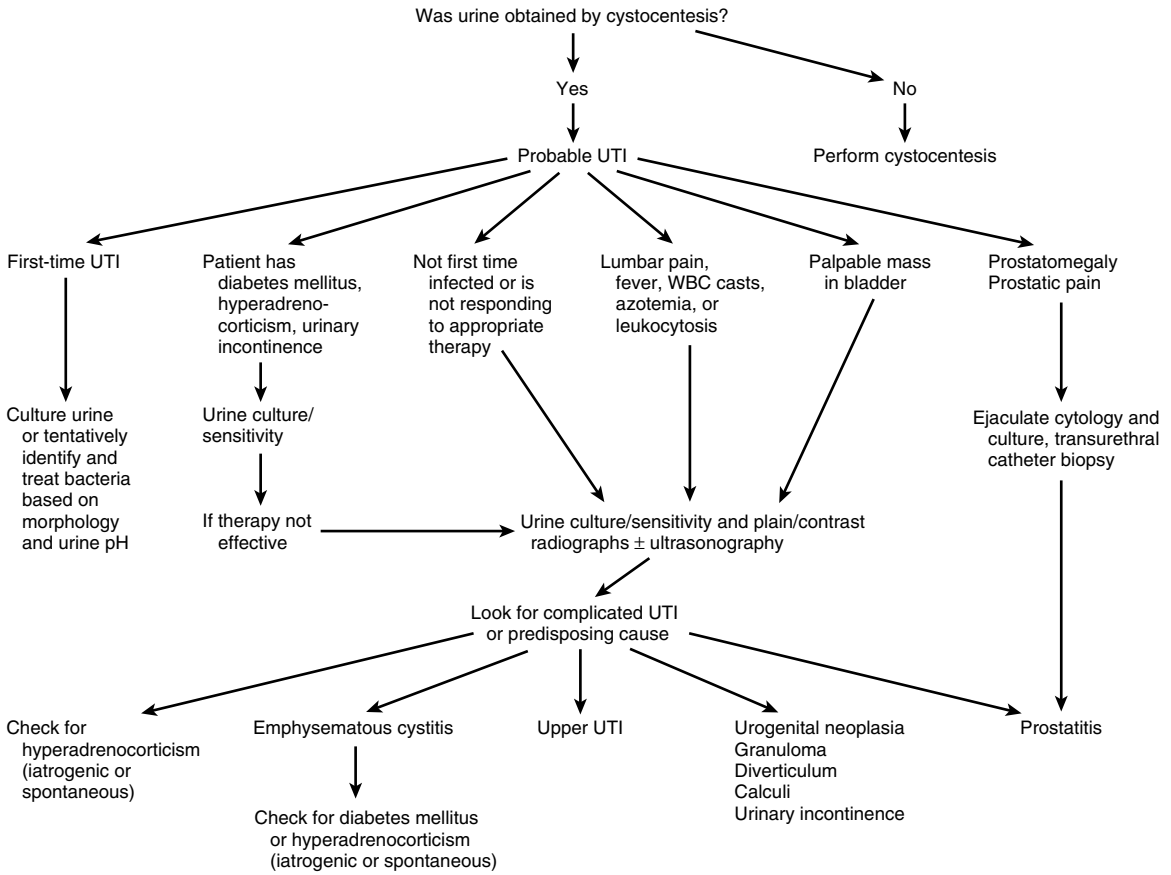

FIGURE 7-6. Diagnostic approach to bacteriuria in dogs and cats. UTI = urinary tract infection; WBC = white blood cell.

the likely organism and choose appropriate therapy. If a UTI persists or recurs after treatment, however, urine culture is mandatory. Recurrent UTIs are indications for survey and contrast radiographs, ultrasonography, and ejaculate cytology and culture in intact male dogs. Careful search must be made for underlying causes such as inappropriate therapy (i.e., drug resistance), lack of drug administration by the owner, repeated or indwelling urinary catheterization, neoplasia, partial obstruction, pyelonephritis, prostatitis, calculi, granulomas, diverticuli, incontinence, polyuria, urine retention, diabetes mellitus, and hyperadrenocorticism (Table 7-6).

TABLE 7-5. Presumptive Identification of Bacteria Based on Urinalysis Findings (Culture Should be Used for Confirmation)

URINE pH	BACTERIAL CHARACTERISTICS	LIKELY ORGANISM
Acid	Rods	*E. coli*[*]
Acid	Cocci	Entreococci/streptococci
Alkaline	Rods	*Proteus* spp.
Alkaline	Cocci	Straphylococci

[*]Other gram-negative rods, such as *Klebsiella, Pseudomonas,* and *Enterobacter,* are also possible. *E. coli* is placed in the table because it is the most common cause of urinary tract infection.

Other Cells

Analysis • Microscopic examination of urine sediment is used to look for other cells. A few large and small round cells may be visible in the urine sediment of normal animals.

Causes of Other Cells • Neoplastic cells are occasionally found in the urine of patients with malignancies (e.g., transitional cell carcinoma) of the bladder or urethra. If neoplastic cells are being sought in urine, a large volume of fresh urine should be immediately centrifuged, the sediment smeared on a slide and allowed to dry, and the slide stained with new methylene

TABLE 7-6. Causes of Persistent or Relapsing Urinary Tract Infections and Methods of Diagnosis

CAUSE	MEANS OF DIAGNOSIS
Lack of owner compliance in drug administration	History (check for leftover medications)
Upper urinary tract infection	Excretory urogram showing dilated pelvis, culture urine from renal pelvis, white blood cell casts, ultrasonography
Calculi	Survey and/or contrast radiographs, ultrasonography, cystoscopy
Prostatitis	Ejaculate cytology and culture, prostatic aspirate, prostatic biopsy, ultrasonography
Neoplasm	Cytology of urine sediment, contrast radiographs, biopsy, ultrasonography, urethrocystoscopy
Diverticulum	Positive-contrast radiographs
Granuloma	Contrast radiographs, urethrocystoscopy, biopsy
Urinary incontinence or urine retention due to any cause	History, physical examination, determination of residual urine volume
Decreased resistance to infection	Hyperadrenocorticism, diabetes mellitus (see Chapter 8)
Urinary catheterization	History, physical examination

blue (NMB) or Wright's-Giemsa. Swelling and early degeneration of cells exposed to urine are common, as are reactive changes because of cystitis. These changes may mimic malignancy (i.e., large or atypical nucleus and nucleolus). Radiographic contrast agents may produce similar changes. If urethral or cystic neoplasia is suspected but either no abnormal cells are found in the urine or uncertainty exists as to the nature of the cells seen, a bladder wash or catheter biopsy procedure is preferred to obtain a specimen suitable for cytologic evaluation.

Rarely, funguria occurs in blastomycosis and disseminated aspergillosis. Other fungi and yeasts are usually contaminants, although infection can occur with *Candida albicans* and *Torulopsis* spp.

Sperm are normally visible in the urine of intact males.

Cylindruria

Analysis • Microscopic examination of urine sediment is used to look for cylindruria.

Normal Values • 0 to 2 hyaline or granular casts/lpf in moderately concentrated urine in otherwise normal animals.

Danger Values • None.

Artifacts • Casts disintegrate if urine is stored too long (i.e., hours) or subjected to vigorous mixing or handling. Casts are less commonly visible in alkaline urine.

Causes of Cylindruria • Casts support a diagnosis of renal disease; absence of casts does not eliminate renal disease. The type of cast provides some information about the disease process. Number of casts is not correlated with reversibility or irreversibility of underlying disease.

Hyaline casts may be found during diuresis, after correcting dehydration, or in patients with proteinuria. Granular casts are composed of degenerating cells, proteins, and other substances. Distinguishing coarse granular casts from fine granular casts is not useful. These casts are associated with diseases causing renal tubular epithelium degeneration and necrosis. Cells that cause these casts are probably renal tubular epithelial cells; however, they may also be degenerating WBCs. Waxy casts represent older, degenerate granular casts. Broad casts are wide hyaline, granular, or waxy casts. They are thought to be wide because of formation in collecting ducts or dilated renal tubules. Renal tubular cell casts are often associated with granular casts and indicate active renal tubular injury. WBC casts signify renal tubulointerstitial inflammation. This type is rare, and few patients with pyelonephritis have them. Therefore absence of this cast does not exclude pyelonephritis. RBC casts are rare and signify hemorrhage into renal tubules or severe glomerular injury, allowing RBCs to enter the tubules (e.g., glomerulonephritis, vasculitis, renal infarction).

Crystalluria

Analysis • Microscopic examination of urine sediment is used to look for crystalluria. Crystal habit (i.e., the characteristic shapes of mineral crystals) is used as an index of crystal composition (Table 7-7); however, microscopic identification of urine crystals is imperfect because their appearance is altered by numerous factors. Definitive identification of crystal composition requires special analyses.

TABLE 7-7. Crystals That May Be Found In Canine and Feline Urine

NAME	DESCRIPTION	SIGNIFICANCE
Struvite (magnesium ammonium phosphate)	Colorless prisms with 3-6 sides (coffin lid)	Common in mildly acidic to alkaline urine in normal dogs and cats; may be associated with struvite calculi and infection with urease-producing bacteria
Calcium oxalate (monohydrate)	Dumbbells or small spindles	May be normal, due to ethylene glycol intoxication, or associated with oxalate calculi (Color Plate 5D)
Calcium oxalate (dihydrate)	Colorless envelopes or small stars	May be normal, due to ethylene glycol intoxication, or associated with oxalate calculi
Calcium phosphate	Prisms (long) or amorphous	May be normal or associated with calculi
Ammonium urate	Yellow-brown "thorn apples"	Normal in Dalmatians and English bulldogs; associated with hepatic insufficiency and portosystemic shunts; may be associated with urate calculi
Uric acid	Yellow to yellow-brown prisms, diamonds, or rosettes	Same as ammonium urate
Bilirubin	Golden-yellow to brown needles or granules	May be present in normal dogs with concentrated urine or due to bilirubinuria
Cystine	Colorless, flat hexagonal plates	Due to cystinuria; may be associated with calculi
Cholesterol	Colorless, flat, notched plates	May be found in normal dogs and cats
Hippuric acid	Prisms (4-6 sides) with rounded corners	Uncertain; have been confused with calcium oxalate monohydrate crystals
Sulfonamide	Clear to brown eccentrically bound needles in sheaves	Associated with sulfonamide administration

Artifacts • Crystalluria means the urine specimen is oversaturated with crystallogenic substances; however, numerous variables influence crystalluria. The significance of crystalluria is easily misjudged if these factors are not considered. *In vivo* variables include urine concentration, urine pH, amount and solubility of crystalloids, and excretion of medications or diagnostic agents. *In vitro* variables include temperature, evaporation, pH, and technique of specimen preparation.

Causes of Crystalluria • When crystal type is important (because of current or prior occurrence of calculi or because one suspects a portosystemic shunt or ethylene glycol toxicity [see Color Plate 5D]), fresh specimens should be examined. Number, size, and structure of crystals should be evaluated, as well as their tendency to aggregate.

Detection of ammonium urate crystals in breeds besides Dalmatians and English bulldogs may suggest hepatic insufficiency (i.e., portosystemic shunt). Calcium oxalate crystals in animals in acute renal failure suggest ethylene glycol ingestion.

Crystalluria often causes concern about urolithiasis. Evaluation of urine crystals may aid in detecting conditions that predispose to urolith formation, estimating mineral composition of existing uroliths, and evaluating effectiveness of therapy intended to dissolve uroliths or prevent their reformation. Crystalluria must not be the sole criterion for assessment of stone composition when uroliths are present, however. Animals with crystalluria do not necessarily form uroliths, and finding crystalluria is not always an indication for treatment. For example, dogs and cats normally excrete a large amount of ammonium magnesium phosphate (i.e., struvite). With urine pH greater than 6.5, this normal excretion begins to become visible as struvite crystals. The higher the urine pH, the more crystals become evident. Thus struvite crystalluria is normal in most dogs and cats. Urolithiasis becomes a risk when urine pH remains consistently alkaline, usually from infection with urease-producing bacteria (dogs) or when urine is very concentrated in association with a urine pH greater than 6.5 (cats).

WATER DEPRIVATION TESTING

Indication • Water deprivation testing is indicated in selected patients with severe pu-pd: generally those that are hyposthenuric and in whom most causes of pu-pd have been excluded by history, physical examination,

CBC, urinalysis, biochemical profile (i.e., azotemic renal failure, hepatic failure, diabetes mellitus, hypercalcemia, hyponatremia, hypokalemia), and endocrine testing where indicated (i.e., hyperthyroidism, hyperadrenocorticism, hypoadrenocorticism) (see Table 7-1). After excluding these diseases, water deprivation testing can distinguish apparent psychogenic polydipsia from central diabetes insipidus or nephrogenic diabetes insipidus (Figure 7-7).

Advantage • Water deprivation testing is specific for psychogenic polydipsia when more common causes have been eliminated.

Disadvantages • The test is not able to differentiate between many common medical causes of pu-pd, and close monitoring is needed to avoid morbidity and mortality.

Analysis • This test is performed only in nonazotemic, euhydrated patients after taking a thorough history, performing a complete physical examination, and evaluating a CBC, biochemical profile, and urinalysis. One must eliminate drugs and diets that cause pu-pd (e.g., diuretics, glucocorticoids, anticonvulsants, excessive thyroid supplementation, low-protein or high-salt diets). Changes in environment are also possible.

An abrupt water deprivation test is typically used at The University of Georgia. The patient is hospitalized for the test, which is begun with an overnight fast during which water intake is not limited. Urine first voided in the morning is collected and specific gravity is measured (because urine is most likely to be concentrated at this time of day). A portion of the urine and a blood sample are saved for measurement of osmolality. The animal is walked to encourage defecation and complete urination. The bladder is palpated to be sure it has been emptied. The animal is then accurately weighed. A 5% decrease in body weight is calculated and becomes the target weight at which point the test will be ended. The test is also ended if the urine specific gravity reaches 1.035. All access to water is removed. Fasting is continued. Urine is collected, specific gravity measured, and the animal weighed every 2 to 4 hours, depending on

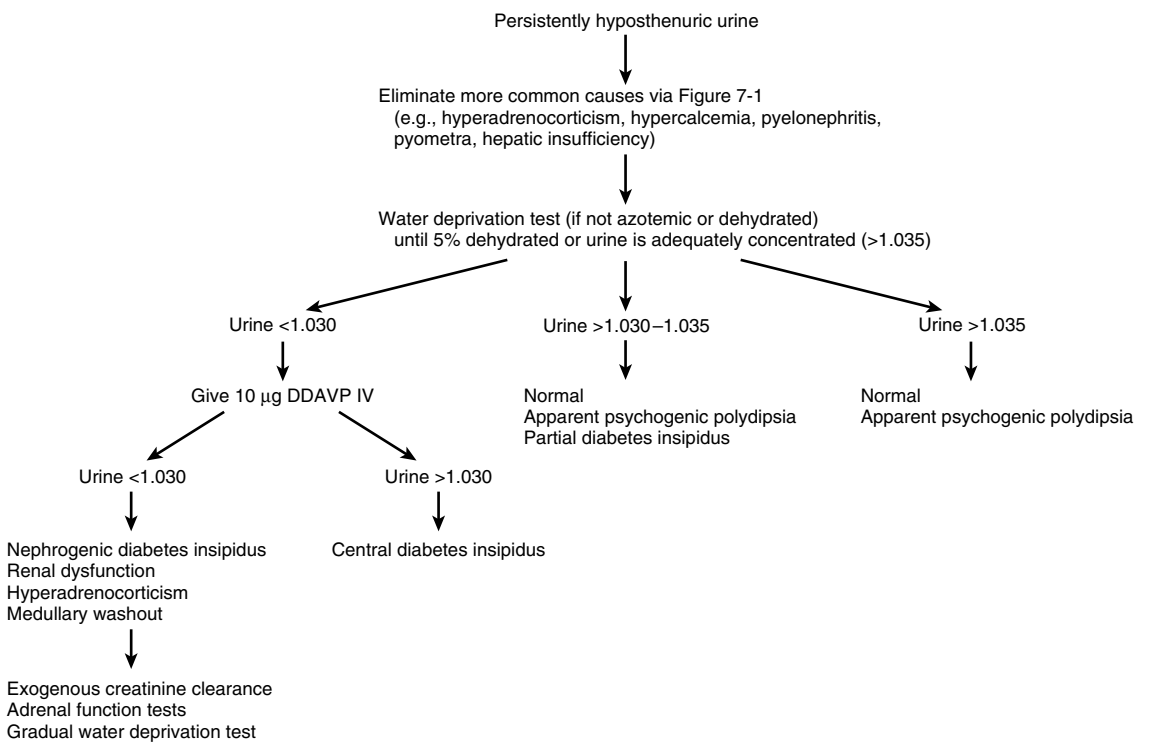

FIGURE 7-7. Use of water deprivation and antidiuretic hormone (ADH) response testing for differentiation of causes of polyuria-polydipsia, especially those which result in hyposthenuric urine.

the rate of weight loss. Once the target weight or specific gravity is reached, samples are collected for urine and plasma osmolality.

A practical problem with water deprivation testing is that the duration of the test is unpredictable and one is often faced with night approaching and an animal that has neither reached 5% dehydration nor experienced concentration to 1.035. In this situation, one can transfer the animal to a facility with overnight care so that sampling can continue or one can provide the animal with a maintenance water amount (calculated at 2.75 ml/kg/hour that the animal is to be unobserved). The next morning the animal is weighed, specific gravity is measured, water is again withdrawn, and the test is continued until the original target weight indicating 5% dehydration or a specific gravity of 1.035 is reached. With a prolonged test, dry food can be fed.

Several methods have been described for performing a more gradual water deprivation test. All methods involve first measuring the animal's water intake over 24 hours. Body weight is also measured, and a 5% body weight loss calculated. In one method, the amount of water offered is gradually restricted to an estimated normal maximal water intake (i.e., 60 ml/kg/day) over 3 days and then the animal is completely deprived of water. In another method, volume of water given daily is reduced each day from the initial measured amount by an amount equal to 2% of the initial body weight. For example, if the animal weighed 10 kg and drank 1.5 L/day, the amount offered each day would be reduced by 0.2 L; the animal would be given 1.3 L the first day, 1.1 L the second day, and so forth. Regardless of method, urine specific gravity and the animal's weight should be monitored each day initially and then more frequently as the target weight is approached. The test is interpreted the same way as the abrupt test. The reason for performing a gradual rather than an abrupt test is concern that medullary washout of solute secondary to prolonged polyuria will prevent urine concentration during abrupt water deprivation. Usually, gradual water deprivation tests are performed if results of the abrupt test are ambiguous.

Normal Values • Of normal cats and dogs, 95% experience concentration of urine to a specific gravity of 1.048 before they lose 5% of their body weight. Normal dogs and cats require several days to reach this level of dehydration. Normal animals reach the target specific gravity of 1.035 before they reach the target degree of dehydration. A pu-pd patient with normal response to water deprivation most likely has psychogenic polydipsia; however, some dogs with hyperadrenocorticism respond to water deprivation. If urine specific gravity does not reach 1.030 with 5% dehydration, the animal's response is definitively abnormal. Values between 1.030 and 1.035 are a questionable response but may indicate a degree of medullary washout or partial central or nephrogenic diabetes insipidus. Measurement of serum osmolality can confirm that dehydration has occurred by the change from the beginning to the end of the test. A 1% to 2% increase in serum osmolality induces maximal release of ADH. Urine osmolality changes can be used to confirm the specific gravity measurements.

Danger Values • *Warning:* Failure to closely monitor patients may allow life-threatening hypernatremic dehydration, especially in small patients or those with severe pu-pd as the result of hyposthenuric disorders (e.g., diabetes insipidus). Failure to perform a urinalysis, CBC, and biochemical profile before the test can lead to destabilization of a serious medical problem (e.g., azotemic renal failure, hypercalcemia, diabetes mellitus, hepatic failure).

Artifacts • Urine specific gravity may be falsely increased by contamination, suggesting concentration. The accuracy of the refractometer should be confirmed.

ANTIDIURETIC HORMONE RESPONSE TESTING

Indication • If urine is not adequately concentrated with water deprivation, ADH response testing is used to differentiate central from nephrogenic diabetes insipidus.

Analysis • The clinician can use several methods to perform this test. At The University of Georgia, a synthetic analog of vasopressin (i.e., deamino D-arginine vasopressin [DDAVP]) is used most frequently. The animal is allowed to reestablish normal hydration before testing, and water is not withheld during testing. Ten micrograms of DDAVP are injected IV, and urine specific gravity is measured 1, 2, 4, 6, 8, 12, and 24 hours later. In another method, ADH response testing is conducted

immediately after water deprivation and with continued water deprivation. Aqueous vasopressin is administered intramuscularly at a dose of 0.55 IU/kg, with a maximum of 5 IU. The bladder is emptied and urine specific gravity measured every 30 minutes for 2 hours. Serum and urine osmolalities are also collected every 2 hours. The animal is then gradually reintroduced to ad lib water over the next 2 hours.

Normal Values • Exogenous administration seems less effective in stimulating a maximal response than water deprivation. Urine specific gravity greater than or equal to 1.030 or urine/plasma osmolality of greater than 3:1 (typically > 5:1) indicates renal responsiveness to ADH. Such a response in an animal that did not respond to water deprivation highly suggests complete or partial central diabetes insipidus (see Figure 7-7). Partial central diabetes insipidus and hyperadrenocorticism can be difficult to differentiate, because both can cause some response (but less than normal) to water deprivation and some responsiveness to exogenous vasopressin. Adrenal function tests may be necessary to differentiate these two conditions.

Animals not responding normally to water deprivation or ADH most likely have preazotemic renal failure or primary or secondary nephrogenic diabetes insipidus. Animals with nephrogenic diabetes insipidus usually have hyposthenuric urine before the test, whereas patients with renal failure usually have isosthenuric or mildly concentrated urine. Note that hyperadrenocorticism causes secondary nephrogenic diabetes insipidus; thus responses of such animals to water deprivation and ADH response testing can mimic the responses in other diseases causing pu-pd (see Chapter 8).

Danger Values • *Warning:* Unlimited access to water in a dehydrated animal to which ADH is administered could lead to water intoxication if the patient responds to ADH.

ANURIA-OLIGURIA

Anuria and oliguria necessitate aggressive diagnostic efforts because the prognosis is guarded to poor unless appropriate therapy is quickly begun. The clinician's immediate diagnostic aims are to simultaneously determine the presence of life-threatening secondary changes and the cause of the oliguria (Figure 7-8). One must first determine from history and physical examination if the patient has a urinary obstruction or rupture, severe dehydration, or any likelihood of nephrotoxins. Often passing a urethral catheter and checking for abdominal fluid can eliminate obstruction and rupture. If abdominal fluid is present, its creatinine concentration should be compared with that of the serum (see Chapter 10). Urine for urinalysis and blood for CBC and chemistry profile should be obtained (at least serum sodium, potassium, calcium, phosphorous, Tco_2, anion gap, glucose, BUN, and creatinine). The clinician should check for hyperkalemia (chemistry analysis or electrocardiogram [ECG], although the ECG is not as sensitive or specific) and severe acidosis (blood gas preferred, but Tco_2 is useful). Severe azotemia, hyperkalemia, and acidosis should be treated as soon as they are identified. Dehydration should be corrected, but first the clinician should try to collect a urine sample to determine specific gravity.

If UTI is present, a urine culture should be obtained. If an indwelling urinary catheter is being used for patient management, antibiotic therapy for the UTI should be avoided unless the clinician finds evidence of systemic or renal infection.

Ethylene glycol intoxication must be considered in any dog or cat with acute oliguric renal failure, regardless of "lack of exposure." Calcium oxalate crystalluria often occurs within the first 24 hours of ingestion. The elongated crystals found in association with ethylene glycol toxicity were formerly thought to be composed of hippuric acid but are calcium oxalate monohydrate. In early stages of intoxication, patients have severe metabolic acidosis, central nervous system (CNS) signs (i.e., ataxia, seizures), and hypocalcemia. Hyperosmolality and increased osmolal and anion gaps (see Chapter 6) suggest ethylene glycol intoxication. If ethylene glycol is even a remote possibility, a blood test for ethylene glycol should be performed (see Chapter 17). Anuria-oliguria and uremia usually do not occur for 1 to 4 days after ingestion, depending on the amount ingested. By that time, calcium oxalate crystalluria and hypocalcemia may have resolved. Hyperkalemia is not invariable. Ultrasonography may detect marked renal hyperechogenicity because of deposition of calcium oxalate crystals. Renal biopsy may be needed to confirm the diagnosis once uremia has developed.

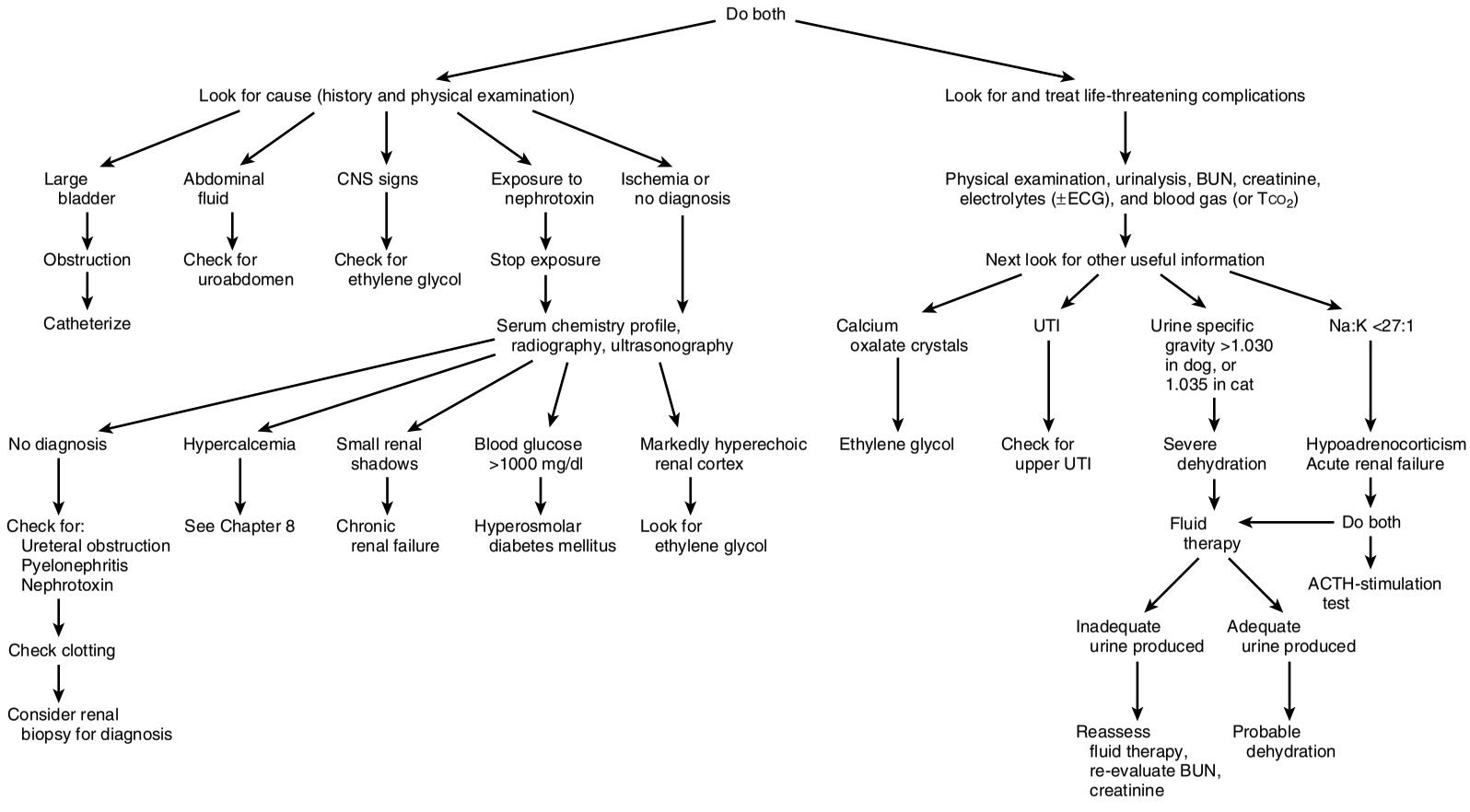

FIGURE 7-8. Diagnostic approach to anuria or oliguria in dogs and cats. ACTH = adrenocorticotropic hormone; BUN = blood urea nitrogen; CNS = central nervous systems; ECG = electrocardiogram; UTI = urinary tract infection.

Hypoadrenocorticism may mimic acute renal failure and hypercalcemic nephropathy. Hyponatremia, hyperkalemia, and decreased Na:K ratio that occur in hypoadrenocorticism can occur in acute renal failure, and some hypoadrenal patients do not have normal renal concentrating ability. Therefore, if serum electrolyte concentrations suggest hypoadrenocorticism, one should begin appropriate fluid therapy and perform an ACTH-stimulation test. Most patients with hypoadrenocorticism produce urine in response to IV fluids. If hypoadrenocorticism is strongly suspected, glucocorticoid therapy should commence after the ACTH test is completed. Most dogs with hypoadrenocorticism respond rapidly to fluid and glucocorticoid support. Glucocorticoids should not be used indiscriminately in azotemic animals, because the drugs can worsen both azotemia and uremic enteritis.

Renal ischemia from any cause (including dehydration and hypoadrenocorticism) may produce oliguria. Most of these dogs and cats produce urine once they are properly rehydrated. It can be difficult to distinguish if severe, prolonged ischemia has caused significant renal parenchymal destruction, however. If the cause of the oliguria is uncertain and urine production remains inadequate despite appropriate fluid therapy, fractional urine sodium excretion (see Chapter 6) may help determine if renal tubular necrosis has occurred.

If oliguria persists despite appropriate IV fluid administration and cause is still uncertain, bilateral ureteral obstruction (rare) or unilateral ureteral obstruction in an animal with only one functional kidney must be considered. Abdominal ultrasonography is useful for eliminating these possibilities. Excretory urograms are contraindicated in markedly azotemic animals because insufficient dye is excreted, and the contrast agent may worsen renal failure.

Hypercalcemia usually causes pu-pd, but in severe or longstanding cases renal calcification may be causing oliguria. Hyperosmolar diabetes mellitus uncommonly causes acute oliguric renal failure. The blood glucose level in these patients is often greater than 1000 mg/dl.

Patients with anuric and oliguric renal failure of unknown cause not responding well to initial therapy or requiring prolonged, expensive therapy should undergo a clotting screen and a renal biopsy for diagnosis and prognosis. Biopsy is usually performed using laparoscopy, ultrasonography, or keyhole technique in dogs and percutaneously in cats.

AZOTEMIA-UREMIA

Azotemia (i.e., above-normal BUN or serum creatinine concentrations) and uremia (i.e., azotemia plus clinical signs such as lethargy, depression, reduced appetite, vomiting, weight loss) are caused by decreased glomerular filtration. The clinician must remember three things. First, a mild increase in BUN or serum creatinine concentration signifies a substantial decrease in glomerular filtration (i.e., >75% decrease in GFR). Second, such a substantial decrease in GFR can be caused by prerenal (e.g., severe dehydration) and postrenal (e.g., urethral obstruction) causes and by renal diseases. Third, factors unrelated to GFR can also mildly affect these tests (especially BUN).

There can be significant renal disease without azotemia. A complete urinalysis may document renal disease (i.e., proteinuria, glucosuria with normoglycemia, casts, reduced concentrating ability) before azotemia occurs (aminoglycoside nephrotoxicity typically causes isosthenuria, proteinuria, glucosuria, or cylindruria before causing azotemia). Therefore, patients treated with such drugs should be periodically evaluated with both urinalysis and serum creatinine.

A simultaneous urinalysis must be performed to allow accurate evaluation of serum creatinine and BUN. The first step in evaluating azotemia is to decide whether it is prerenal, renal, or postrenal (Figure 7-9) (Table 7-8; see discussion of azotemia in Blood Urea Nitrogen).

BLOOD UREA NITROGEN

Indications • BUN can be used to screen for renal function as part of a general health profile or in any ill animal (especially those with vomiting, weight loss, chronic nonregenerative anemia, pu-pd, anuria-oliguria, chronic UTI, proteinuria, or dehydration). Serum creatinine should be measured simultaneously.

Advantages • Tests for BUN concentration are available and easy to use.

Disadvantages • BUN concentration is affected by extrarenal factors (sometimes this is an advantage when assessing owner compliance with recommended reduced-protein diets). In addition, concentration is inversely affected by rate of urine flow.

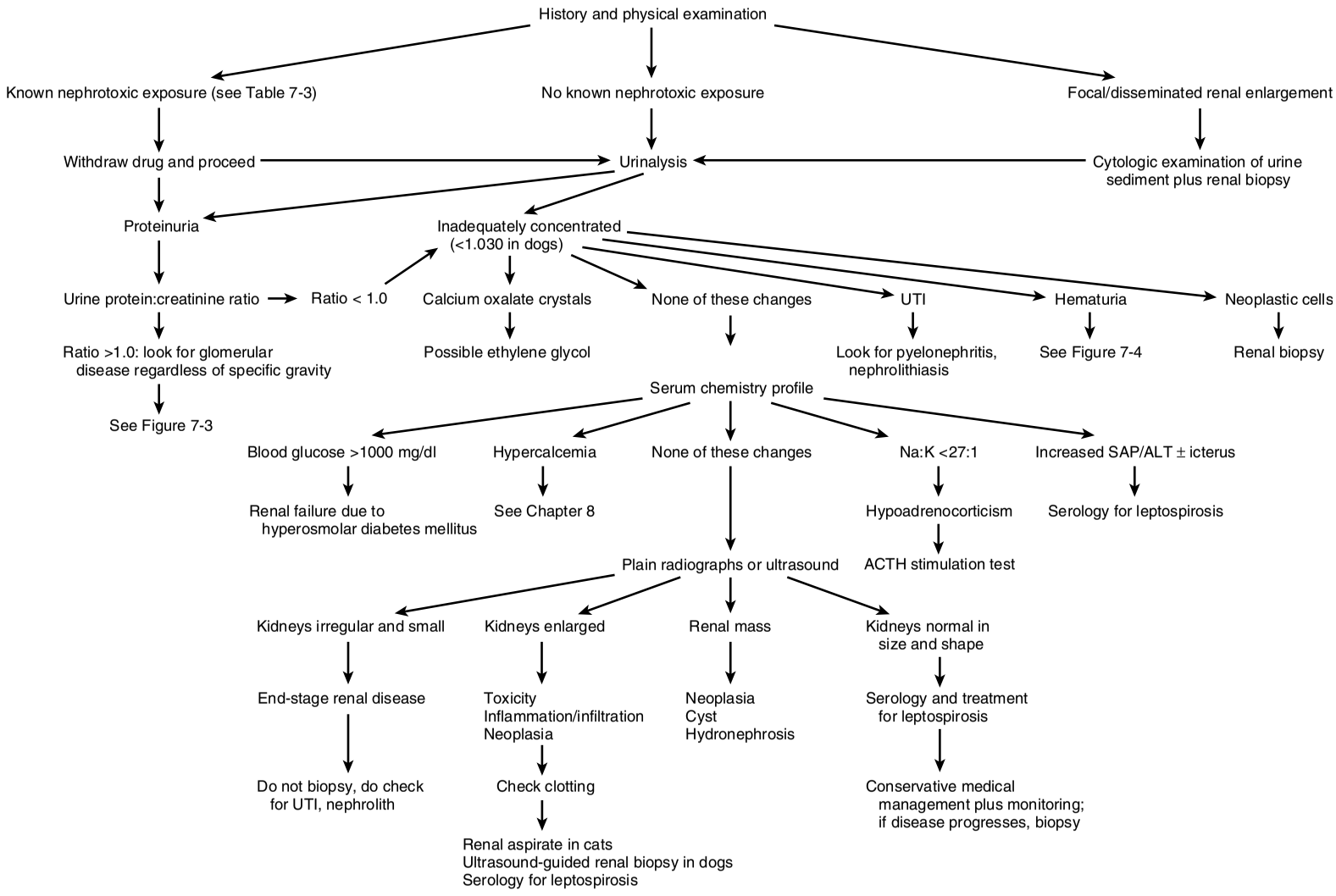

FIGURE 7-9. Diagnostic approach to nonoliguric renal azotemia in dogs and cats without blocked or ruptured urinary tracts. ACTH = adrenocorticotropic hormone; ALT = alanine aminotransferase; SAP = serum alkaline phosphatase; UTI = urinary tract infection.

TABLE 7-8. Distinguishing Characteristics of Prerenal, Renal, and Postrenal Azotemia in Dogs and Cats

Prerenal azotemia	Urine specific gravity > 1.030 (dogs); no definitive specific gravity for cats (see below). NOTE: Significant proteinuria with a benign sediment may be due to primary glomerular disease, in which case a concentrated urine specific gravity does not rule out primary renal disease.
Renal azotemia	Urine specific gravity 1.008-1.030 (dogs) or 1.008-1.035 (cats); some cats in early renal failure have urine specific gravity of > 1.035, whereas dogs in renal failure may have urine specific gravity of 1.006-1.007. Patient may be polyuric, oliguric, or anuric.
Postrenal azotemia	Animal cannot urinate because of urethral obstruction or urine is emptying into the abdomen because of a ruptured urinary tract; ureteral or renal pelvic obstruction, either bilateral or unilateral, if there is only one functional kidney. Urine specific gravity may be any value.

Analysis • BUN is measured in serum or plasma (heparin or ethylenediaminetetraacetic acid [EDTA]) by spectrophotometric, "dry reagent" reflectance meter, and ammonia-sensitive electrode methods, as well as by placing one drop of fresh whole blood on a dipstick. Different methods give comparable results, except for the dipstick (which provides only a crude estimate).

Normal Values • Dogs and cats, generally 10 to 30 mg/dl.

Danger Values • Urea itself is nontoxic; however, marked increases are associated with uremia in which acid-base, fluid, and electrolyte disorders may become life-threatening.

Artifacts • See Introduction to Serum Chemistries: Artifacts in Biochemical Determinations.

Drug Therapy That May Alter Results • Decreased BUN may be the result of drugs causing marked pu-pd. Increased BUN may be caused by corticosteroids, arginine, and nephrotoxic drugs (see Table 7-3).

Causes of Decreased BUN • BUN is decreased by decreased production (i.e., hepatic insufficiency, dietary protein restriction) or increased excretion (i.e., polyuric conditions, overhydration, late pregnancy). Decreased BUN may be an indication for hepatic function tests (see Chapter 9).

Causes of Increased BUN • Increased BUN requires concurrent pretreatment urinalysis for proper interpretation. Serum creatinine concentration should also be measured. If the serum creatinine concentration is normal, extrarenal factors affecting BUN must be considered (Table 7-9). These extrarenal factors usually cause only mild changes unless underlying renal disease is present.

If both BUN and creatinine are increased, decreased GFR is established. Decreased filtration, however, may be the result of prerenal causes (e.g., inadequate renal perfusion because of shock, dehydration, poor cardiac output), renal parenchymal disease, or postrenal causes (e.g., urethral or ureteral obstruction; bladder, urethral, or ureteral rupture; see Table 7-8).

Prerenal azotemia is typically associated with urine specific gravities of greater than 1.030 in dogs and 1.035 in cats and similar increases in urine osmolality. Cats with early renal disease may have azotemia and urine

TABLE 7-9. Causes of Incongruities Between BUN and Serum Creatinine Concentrations

INCREASED BUN PLUS NORMAL SERUM CREATININE	INCREASED SERUM CREATININE PLUS NORMAL TO LOW BUN
Early prerenal azotemia (decreased urine flow rate)	
Increased BUN	**Decreased BUN**
High-protein diet	Hepatic insufficiency
Gastrointestinal hemorrhage	Polyuria-polydipsia
Tetracycline or corticosteroid administration	Low-protein diet
Fever	
Severe tissue trauma (?)	
Decreased Creatinine	**Increased Creatinine**
Decreased muscle mass (severe cachexia needed to cause significant changes)	Myositis/muscle trauma *(unlikely)* Cooked meat diet (mild, transient changes) Ketonemia (falsely increased)

BUN = blood urea nitrogen.

specific gravities of greater than 1.035, however. It is important to obtain pretreatment urine for analysis. If a patient is receiving fluid therapy or drugs that alter renal concentrating ability (e.g., diuretics, corticosteroids) or has another disease inhibiting renal tubular function (e.g., hypercalcemia), urine specific gravity may be inappropriately decreased, making it appear that renal azotemia is present when in fact *prerenal* disease is present. Occasionally, a clue may be the finding of hyposthenuric urine (i.e., specific gravity < 1.008). The ability to dilute the glomerular filtrate indicates normal renal tubular function up to the distal tubule and collecting duct. Some dogs with renal failure may have mildly hyposthenuric urine (i.e., 1.006 to 1.007), however.

A special case involves significant proteinuria in urine of any specific gravity. Glomerular lesions may impair glomerular filtration and cause azotemia despite adequately concentrated urine. Called *glomerulotubular imbalance,* the urine is concentrated because glomerular lesions have not yet resulted in sufficient tubular injury to impair renal concentrating ability.

Inadequately concentrated urine (i.e., urine specific gravity of 1.008 to 1.029) plus an increased BUN and serum creatinine concentration suggests primary renal disease; although other diseases that can result in dehydration and decreased renal concentrating ability may appear similarly (Table 7-10). Many patients with renal azotemia have chronic renal disease of unknown cause. Hypercalcemia, pyelonephritis, drug nephrotoxicity (e.g., aminoglycoside, amphotericin B), leptospirosis, and hyperosmolar diabetes mellitus, however, are causes of renal azotemia that, although potentially life-threatening, may be resolved with early diagnosis and appropriate therapy. Hypoadrenocorticism may produce identical urine specific gravity, BUN, and serum creatinine values that are not caused by morphologic renal lesions but are reversible with proper therapy. Therefore, after a complete urinalysis is performed, renal azotemia is an indication for carefully reviewing history, physical examination, CBC, and biochemical profile (e.g., serum sodium, potassium, calcium, total protein, albumin, glucose, Tco_2 values; see Figure 7-9). Although of poor sensitivity, survey abdominal radiographs may reveal focal or diffuse renomegaly, decreased renal size, or nephroliths. Ultrasonography can also be used to evaluate the kidneys.

Hyperechogenicity is common in both acute and chronic renal diseases; however, marked hyperechogenicity suggests ethylene glycol toxicity. Excretory urography may be useful in mild-to-moderate renal azotemia to document pyelonephritis, nephrolithiasis, ureterolithiasis, and renal size and shape; however, care must be taken to avoid exacerbating renal disease.

Blood Urea Nitrogen in Abdominal Fluids • Finding a significantly higher urea concentration in abdominal fluid than in blood suggests urinary tract rupture; however, urea readily diffuses across the peritoneal membrane. Forty-eight hours after bladder rupture, urea concentrations may be similar between abdominal fluid and serum. Therefore, measurement of fluid creatinine is preferred.

TABLE 7-10. Diseases/Conditions That May Result in Azotemia with a Urine Specific Gravity Between 1.008 and 1.029

Acute or chronic renal insufficiency
E. coli septicemia/pyometra/prostatic abscessation
Pyelonephritis
Hypoadrenocorticism
Hypercalcemia
Hyponatremia
Hypokalemia
Ketoacidotic or hyperosmolar diabetes mellitus
Hyperadrenocorticism with dehydration
Diabetes insipidus with dehydration
Hepatic failure
Urinary tract obstruction or rupture
Treatment of any prerenal cause of azotemia with fluids or diuretics

CREATININE

Indications • Measurement of creatinine concentration is indicated for the same reasons as for BUN.

Advantages • Serum creatinine concentration is not altered by as many extrarenal factors as BUN or by urine flow rate.

Analysis • Serum creatinine concentration is measured in serum or plasma (heparin) by spectrophotometric or dry reagent reflectance meter methods. These give comparable results.

Normal Values • Dogs and cats, generally less than 1.7 mg/dl.

Danger Values • Same as for BUN.

Artifacts • See Introduction to Serum Chemistries.

Drugs That May Alter Results • Nephrotoxic drugs (see Table 7-3) may increase the serum creatinine concentration.

Causes of Decreased Serum Creatinine • Decreased serum creatinine may be the result of significant loss of muscle (see Table 7-9) or pregnancy (which increases cardiac output and subsequently GFR).

Causes of Increased Serum Creatinine • See Figure 7-9 and Table 7-9. Feeding cooked meat may increase serum creatinine by less than 1 mg/dl. Acute myositis and severe muscle trauma are potential causes, but their significance is uncertain. Decreased glomerular filtration is the major cause of increased serum creatinine concentrations. As for BUN, decreased filtration may be prerenal, renal, or postrenal in origin. Urine specific gravity is essential for differentiating renal and prerenal causes, as described for BUN. An increased serum creatinine concentration is an indication for urinalysis and measurement of BUN. An increased serum creatinine concentration and BUN plus inadequately concentrated urine is an indication for a CBC, biochemical profile (e.g., serum sodium, potassium, calcium, phosphorous, total protein, albumin, glucose, Tco_2), and renal imaging.

Creatinine Concentration in Abdominal Fluid • Abdominal fluid creatinine concentration is useful in diagnosing uroabdomen. An abdominal fluid creatinine concentration substantially greater than serum creatinine is highly suggestive of uroabdomen and an indication for a positive-contrast cystogram or excretory urography.

URINE CREATININE

Indications • Urine creatinine measurement is indicated for calculation of clearance or fractional excretion and in assessing significance of proteinuria (i.e., urine protein: urine creatinine ratio).

Analysis • Urine creatinine is measured by spectrophotometric methods.

URINE FRACTIONAL EXCRETION

Indications • Determination of urine fractional excretion is indicated to assess renal clearance of various substances (i.e., sodium, potassium, calcium, phosphorus, albumin). See respective sections for discussions of the uses of each.

Analysis • The clinician can use the following formula, in which all values are determined on simultaneous blood and urine samples:

$$\frac{\text{Urine substance}}{\text{Plasma substance}} \times \frac{\text{Plasma creatinine}}{\text{Urine creatinine}} \times 100$$

MEASUREMENT OF GLOMERULAR FILTRATION RATE

Indications • Measurement of glomerular filtration rate is indicated in patients with suspected nonazotemic renal disease or patients with renal disease who are in need of serial monitoring.

Analysis • Three tests are readily available and reasonably accurate for the measurement of glomerular filtration rate: (1) endogenous creatinine clearance, (2) exogenous creatinine clearance, and (3) iohexol clearance.

Creatinine Clearance

Analysis • For *endogenous* clearance, a 24-hour urine collection plus a serum sample taken approximately midway through the urine collection is required. Total volume of urine produced in 24 hours is measured, and creatinine concentrations are determined on the serum and a 3 ml aliquot of pooled urine. The clearance is calculated as follows:

$$\frac{\text{Urine volume (ml)} \times \text{Urine creatine (mg/dl)}}{\text{Time (min)} \times \text{Serum creatinine (mg/dl)} \times \text{Wt (kg)}}$$

which gives a value in ml/min/kg.

For *exogenous* clearance, a creatinine solution (i.e., 25 mg/ml) is administered subcutaneously at 75 to 100 mg/kg. A stomach tube is passed, and water equal to 3% of body weight is administered. A urinary catheter is inserted and left in place. The bladder is carefully emptied and rinsed with saline twice at 38 to 40 minutes after the subcutaneous injection. At 40 minutes after injection, a 20-minute

urine collection is begun and a serum sample is obtained at the beginning and end of the urine collection. At 60 minutes, a second 20-minute collection is begun with a final blood sample collected at 80 minutes. Volume of urine for each 20-minute collection plus the three serum and two urine creatinine concentrations (from an aliquot of each 20-minute collection) are determined. The same formula is used as described for endogenous creatinine clearance, except that the serum creatinine concentration used in the calculation is the mean of the three measured serum values.

Iohexol Clearance

The test is performed by administering a single dose of iohexol at 300 mg iodine/kg IV and recording the time to the nearest minute. At 2, 3, and 4 hours after administration, a 3 to 4 ml blood sample is collected, the blood is allowed to clot, and the serum is transferred to a plastic vial (at least 1.2 ml of serum is needed). The exact sampling time must be recorded. The serum samples are then shipped chilled or frozen with frozen gel packs in an insulated container to the Animal Health Diagnostic Laboratory at Michigan State University. The laboratory will then report the glomerular filtration rate.

Normal Values • *Endogenous test:* Dogs, 2.0 to 4.5 ml/min/kg; cats, 1.6 to 3.8 ml/min/kg. *Exogenous and iohexol tests*: Dogs, 3.5 to 4.5 ml/min/kg; cats, 2.6 to 3.2 ml/min/kg.

Danger Values • Not established.

Artifacts • Anything that affects measurement of creatinine may affect creatinine clearance tests (see earlier section on Artifacts under Creatinine). Failure to collect all urine produced during the time period causes significant error in creatinine clearance tests. Measurement of noncreatinine chromogens in plasma as creatinine falsely lowers endogenous creatinine clearance values. Inaccurate recording of dose, time of administration, and actual times of sample collection yields inaccurate calculation of GFR by iohexol clearance.

Drug Therapy and Other Factors That May Alter Results • Decreased GFR may be caused by nephrotoxic drugs (see Table 7-3). State of hydration affects GFR; thus fluid therapy may affect results.

Causes of Decreased Glomerular Filtration Rate • Decreased glomerular filtration rate can be the result of prerenal, renal, and postrenal causes. Because of this, it is important to rule out prerenal (e.g., dehydration, decreased cardiac output) and postrenal causes (e.g., urinary tract obstruction, rupture) before measuring glomerular filtration rate. If these causes are excluded, renal dysfunction is the most likely cause of decreased GFR. Administration of a water load at the beginning of exogenous creatinine clearance avoids subclinical dehydration as a factor. Decreased GFR in a patient without prerenal or postrenal problems and without small or scarred kidneys on renal imaging is reason for blood pressure measurement and renal biopsy.

Causes of Increased Glomerular Filtration Rate • Not significant.

PHOSPHORUS

Serum phosphorus concentrations may increase in patients with decreased GFR. In patients with chronic renal failure, control of hyperphosphatemia is important to help combat renal mineralization and secondary hyperparathyroidism. Evaluation of serum phosphorus is discussed in Chapter 8.

CALCULI

Urinary calculi may cause urethral obstruction (e.g., anuria and oliguria, azotemia, uremia), cystitis and urethritis (e.g., dysuria, hematuria), ureteral or renal pelvic obstruction (e.g., azotemia, uremia), and destruction of renal tissue (e.g., azotemia, uremia). Calculi should be considered in any patient with urinary obstruction, persistent or recurrent UTI, hematuria, or renal failure of unknown cause. Uroliths are diagnosed by physical examination (i.e., bladder or urethral palpation, urinary catheterization), survey or contrast radiographs (some calculi are radiolucent), or ultrasonography. All calculi removed or spontaneously passed should be analyzed quantitatively, and urine should be cultured. Many canine uroliths are struvite and form secondary to urine alkalinization by urease-producing bacteria (principally *Staphylococcus* or *Proteus* spp.), whereas other

types cause UTI secondary to tissue injury. Therefore a diagnosis of UTI and urolithiasis does not mean an infection has caused a struvite calculus unless the urine is alkaline and a urease-producing organism is cultured. Even in this situation, calculus identity is only an educated guess. If therapy does not proceed as expected, calculi should be retrieved and analyzed. Accurate determination of crystalloid composition of calculi is essential for prevention and appropriate medical dissolution.

Calculi Analysis

Indications • All urinary calculi should be analyzed for mineral content.

Advantage • Calculi analysis determines the type of calculi.

Disadvantage • The clinician needs to send calculi to laboratories equipped for proper analysis.

Analysis • Calculi are most commonly analyzed by optic crystallography, x-ray diffraction, and chemical analysis (e.g., the spot test). Less commonly used methods include scanning electron microscopy, electron microprobe, and infrared spectroscopy. Crystallography and diffraction give accurate results, but chemical analysis does not.

Artifacts • Chemical or qualitative analysis (e.g., the spot test) is fraught with inaccuracies. This test should never be used.

Causes of Calculi • This subject is too extensive for discussion in this text. Interested readers are referred to reviews (Osborne and Finco, 1995).

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8

Richard W. Nelson, Grant H. Turnwald, and Michael D. Willard

Endocrine, Metabolic, and Lipid Disorders

CALCIUM

Commonly Indicated • Common indications for serum calcium concentration include patients with lethargy, anorexia, vomiting, constipation, weakness, polydipsia, polyuria (i.e., signs of hypercalcemia), facial pruritus, restlessness, muscle tremors, fasciculations, cramping of rear legs, tetany, or seizures (i.e., signs of hypocalcemia). Other indications include azotemia, diffuse bone disease, and selected abnormalities on an electrocardiogram (ECG), such as prolonged QT interval with a normal QRS complex or unexplained premature ventricular contractions.

Analysis • Serum calcium concentration is measured in serum, heparinized plasma, and urine by colorimetric reactions, spectrophotometric methods, or potentiometry using ion-specific calcium electrodes. Oxalate, citrate, and ethylenediaminetetraacetic acid (EDTA) anticoagulants should not be used, because calcium is bound to these chemicals and becomes unavailable for analysis. Most automated and in-house serum chemistry analyzers measure total serum calcium concentration, which consists of biologically active, ionized calcium (50%), protein-bound calcium (40%), and calcium complexes (10%). In dogs, alterations in the plasma protein concentration may alter the total serum calcium

concentration, yet the ionized calcium levels remain normal. Simple quantitative changes in the albumin and total plasma proteins do not cause hypocalcemia or hypercalcemia in dogs, even though the total serum calcium levels may "appear" low or high on the biochemistry panel. The following formulas can be used to determine the corrected total serum calcium concentration (Meuten et al, 1992):

$$\text{Corrected calcium (mg/dl)} = \text{measured calcium (mg/dl)} - \text{albumin (g/dl)} + 3.5$$

$$\text{Corrected calcium (mg/dl)} = \text{measured calcium (mg/dl)} - [0.4 \times \text{serum protein (g/dl)}] + 3.3$$

The formula based on albumin is preferred because of the stronger relationship between serum albumin and total calcium concentrations. The formulas should not be used in dogs less than 24 weeks of age because high values may be obtained; it should not be used in cats, because no linear relationship exists between serum total calcium and serum albumin and total protein concentrations in cats (Flanders et al, 1989). These formulas yield a rough estimate of the corrected total serum calcium concentration and were developed without verification by serum ionized calcium measurements. Unfortunately, the correlation between "corrected" serum calcium concentration and ionized calcium concentration is weak (Mischke et al, 1996), suggesting that corrected total serum calcium concentrations may not be reliable indicators of calcium homeostasis.

The biologically active, ionized fraction of calcium can be determined directly, thus bypassing the influence of plasma proteins on the total serum calcium concentration. Use of automated equipment with a calcium ion–selective electrode allows accurate measurement of ioinized calcium in blood, plasma, or serum. Samples must be specially handled and the sample pH adjusted (ionized calcium decreases as pH increases) to ensure the accuracy of this method.

Normal Values for Total and Ionized Serum Calcium •

Adults:
Total Ca, 9.0 to 11.5 mg/dl.
Ionized Ca, 1.12 to 1.42 mmol/L.

NOTE: An estimate of total Ca = ionized Ca × 8.

To convert from mg/dl to mmol/L, multiply by 0.25.

Immature: The serum total and ionized calcium concentration can be as much as 1 mg/dl and 0.1 mmol/L higher in young dogs (i.e., < 12 months old), especially in the large and giant breeds, than adults. The serum total calcium concentration does not fluctuate with age in cats, but the serum ionized calcium concentration may be as much as 0.1 mmol/L higher in cats less than 2 years of age, compared with results in older cats.

Danger Values • Total serum Ca less than 7.0 mg/dl (tetany). NOTE: This value depends on the blood pH. The lower the blood pH (i.e., the more acidemic), the lower the calcium can be without causing clinical signs and vice versa. Total serum Ca greater than 16 mg/dl (depending on serum albumin concentration) can result in acute renal failure and cardiac toxicity.

Artifacts • Falsely decreased: increased bilirubin concentrations, laboratory error. Falsely increased: laboratory error, dehydration (mild increase), and lipemia. (See Introduction to Serum Chemistries.)

Drug Therapy That May Alter Serum Calcium Concentration • Mithramycin, EDTA, glucagon, anticonvulsants, citrate, fluoride, glucocorticoids, phosphate-containing enemas, and intravenous (IV) phosphate administration (i.e., potassium phosphate) may cause hypocalcemia.

Vitamin D, cholecalciferol rodenticides, estrogen, progesterone, testosterone, anabolic steroids, acetaminophen, hydralazine, parenteral calcium administration, and excess oral phosphate binders may cause hypercalcemia.

Causes of Hypercalcemia • In dogs, nonparathyroid malignancy (i.e., hypercalcemia of malignancy [HHM]), most notably lymphosarcoma, is the most common cause of hypercalcemia (Table 8-1). Other hemolymphatic malignant tumors (i.e., lymphocytic leukemia, multiple myeloma, myeloproliferative diseases), anal sac apocrine gland carcinoma, and soft tissue tumors metastasizing to bone (e.g., mammary gland adenocarcinoma) may also cause hypercalcemia. Less frequent causes include primary hyperparathyroidism, chronic renal failure, hypoadrenocorticism, and hypervitaminosis D (i.e., cholecalciferol rodenticide toxicity). In the cat, hypercalcemia of malignancy (especially lymphoma and squamous cell carcinoma), chronic renal failure, primary hyperparathyroidism, and

TABLE 8-1. Causes of Hypercalcemia in Dogs and Cats

Hypercalcemia of malignancy
Humoral hypercalcemia
Lymphoma
Apocrine gland adenocarcinoma
Carcinoma (squamous cell, mammary, bronchogenic, prostate, thyroid, nasal cavity)
Hematologic malignancies (bone marrow osteolysis)
Lymphoma
Multiple myeloma
Myeloproliferative disease (rare)
Metastatic or primary bone neoplasia
Hypoadrenocorticism
Chronic and acute renal failure
Hypervitaminosis D
Iatrogenic
Plants
Rodenticides
Primary hyperparathyroidism
Idiopathic hypercalcemia of cats
Granulomatous disease
Sketal lesions
Ostemyelitis
Hypertrophic ostedystrophy
Hypervitaminosis A
Iatrogenic disorders
Excessive calcium supplementation
Excessive oral phosphate binders
Dehydration
Factitious
Lipemia
Postprandial measurement
Young dog (<6 months), large or giant breed
Laboratory error

idiopathic hypercalcemia are the most common diagnoses. Calcium oxalate urolithiasis and consumption of acidifying diets are commonly identified in cats with hypercalcemia; however, their role, if any, in causing hypercalcemia is unknown.

Hypercalcemia should always be reconfirmed, preferably from a nonlipemic blood sample obtained from the dog or cat after a 12-hour fast, before embarking on an extensive diagnostic evaluation. Results of a complete blood count (CBC), serum biochemistry panel, and urinalysis, in conjunction with the history and physical examination findings, often provide clues to the diagnosis (Figure 8-1). Special attention should be paid to the serum electrolytes and renal parameters. Hypoadrenocorticism-induced hypercalcemia occurs in conjunction with mineralocorticoid deficiency; hyponatremia, hyperkalemia, and prerenal azotemia should usually be present. The serum phosphorus concentration is in the lower half of the normal range or low with HHM and primary hyperparathyroidism (Figure 8-2). If the serum phosphorus concentration is increased and renal function is normal, hypervitaminosis D or bone osteolysis from metastatic or primary bone neoplasia is the primary differential. Measurement of serum ionized calcium concentration may help identify dogs and cats with renal failure-induced hypercalcemia; serum ionized calcium concentrations are typically normal or decreased in renal failure and increased in hypercalcemia caused by other disorders.

Hypercalcemia of malignancy and primary hyperparathyroidism are the primary differentials when hypercalcemia and normal-to-low serum phosphorus concentrations are identified. The most common malignancy is lymphoma. A careful review of the history and physical examination findings may provide clues to the diagnosis. Systemic signs of illness suggest hypercalcemia of malignancy. Dogs and cats with primary hyperparathyroidism are usually healthy and clinical signs are mild. The appendicular skeleton, peripheral lymph nodes, abdominal cavity, and rectum should be carefully palpated for masses, lymphadenopathy, hepatomegaly, splenomegaly, or pain on digital palpation of the long bones. Diagnostic tests that are helpful in identifying the underlying malignancy include thoracic and abdominal radiographs; abdominal ultrasound; cytologic evaluation of aspirates of the liver, spleen, lymph nodes, and bone marrow; determination of serum ionized calcium, parathyroid hormone (PTH), and parathyroid hormone related—peptide (PTHrP) concentrations; and cervical ultrasound.

Sternal and hilar lymphadenopathy is common with lymphoma-induced hypercalcemia and can be readily identified with thoracic radiographs. Radiographs of the thorax and abdomen can also be used to evaluate bones; discrete lytic lesions in the vertebrae or long bones suggest multiple myeloma. Hyperproteinemia, proteinuria, and plasma cell infiltration in the bone marrow suggest multiple myeloma. Cytologic evaluation of peripheral lymph node, bone marrow, and splenic aspirates can be helpful in identifying lymphoma; involvement of the peripheral lymph nodes or spleen by lymphoma can be present without causing their enlargement. Ideally the largest lymph node should be evaluated. Normal lymph node, bone marrow, and splenic aspirates do not rule out lymphoma.

Measurement of serum ionized calcium, PTH, and PTHrP from the same blood sample is helpful in differentiating primary

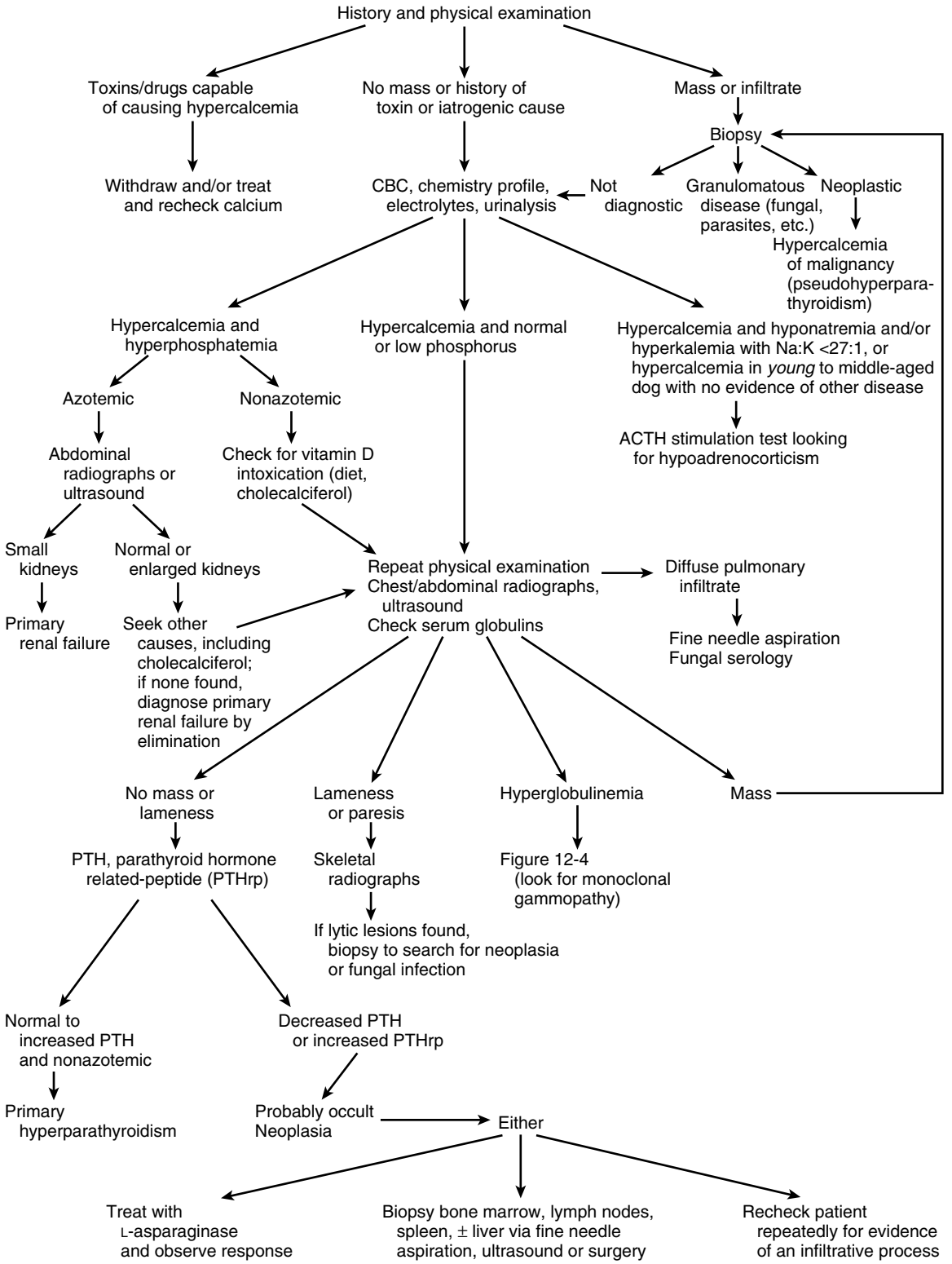

FIGURE 8-1. Diagnostic evaluation of hypercalcemia in dogs and cats. *ACTH,* Adrenocorticotropic hormone; *CBC,* complete blood count; *PTH,* parathyroid hormone.

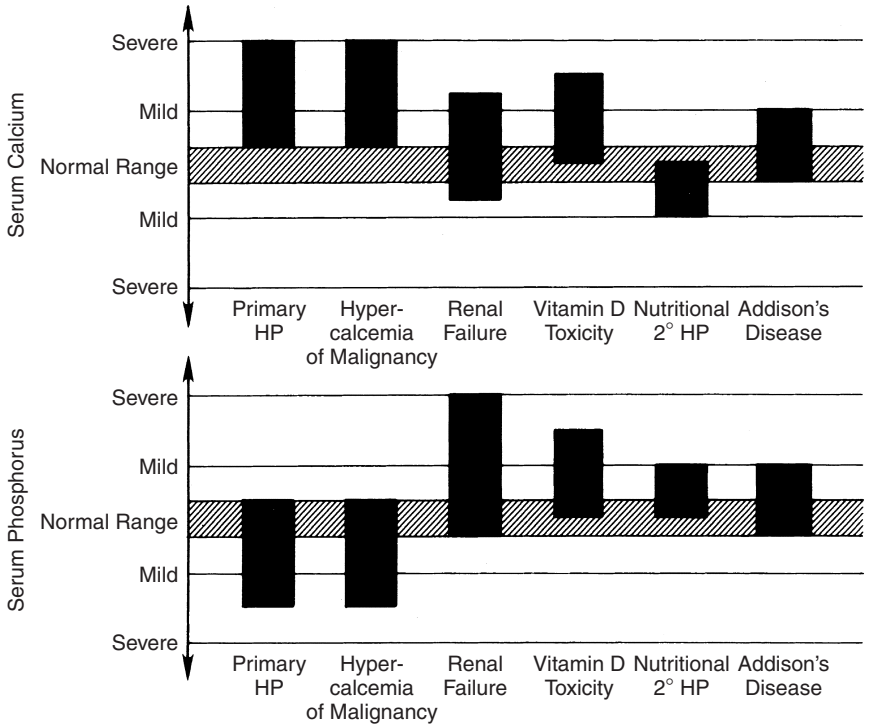

FIGURE 8-2. The range of serum calcium and phosphorus concentrations for the more common causes of hypercalcemia and for hyperparathyroidism in dogs. *HP*, Hyperparathyroidism. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, ed 2, Philadelphia, 1996, WB Saunders.)

hyperparathyroidism from HHM (Figure 8-3). Excessive secretion of biologically active PTHrP plays a central role in the pathogenesis of hypercalcemia in most forms of HHM. Increased serum ionized calcium concentration, detectable serum PTHrP concentration, and nondetectable serum PTH concentration is diagnostic for HHM. Lymphoma is the most common cause for detectable PTHrP concentrations, but other tumors, including apocrine gland adenocarcinoma and various carcinomas (e.g., mammary gland, squamous cell, bronchogenic), can also cause hypercalcemia by this mechanism. In contrast, increased serum ionized calcium, normal to increased serum PTH, and nondetectable PTHrP concentrations are diagnostic of primary hyperparathyroidism. Ultrasonographic examination of the thyroparathyroid complex may reveal enlargement of one or more parathyroid glands. Most parathyroid adenomas measure 4 to 8 mm in diameter, although an occasional parathyroid adenoma will exceed 1 cm. In contrast, the parathyroid glands will be small or undetectable with hypercalcemia of malignancy. Evaluation of the change in serum calcium concentration after L-asparaginase administration should be considered for the patient with hypercalcemia of undetermined cause to rule out occult lymphoma. A marked reduction in serum calcium within 48 hours, usually into the normal range, is strongly suggestive of occult lymphoma.

Idiopathic hypercalcemia is an increasingly common diagnosis in young to middle-aged cats (Midkiff et al, 2000). Hypercalcemia is usually mild (less than 13 mg/dl) and asymptomatic. Serum phosphorus concentration and renal parameters are normal. The cause is unknown. Results of a complete diagnostic evaluation as described previously are unremarkable. Serum PTH concentrations are in the normal range or low; primary hyperparathyroidism has not been confirmed in any of these cats. Nephrocalcinosis and urolithiasis may develop, presumably secondary to increased urinary calcium excretion. Effective treatment has not been identified. Serum calcium concentrations have decreased in some cats after a dietary change to a high fiber

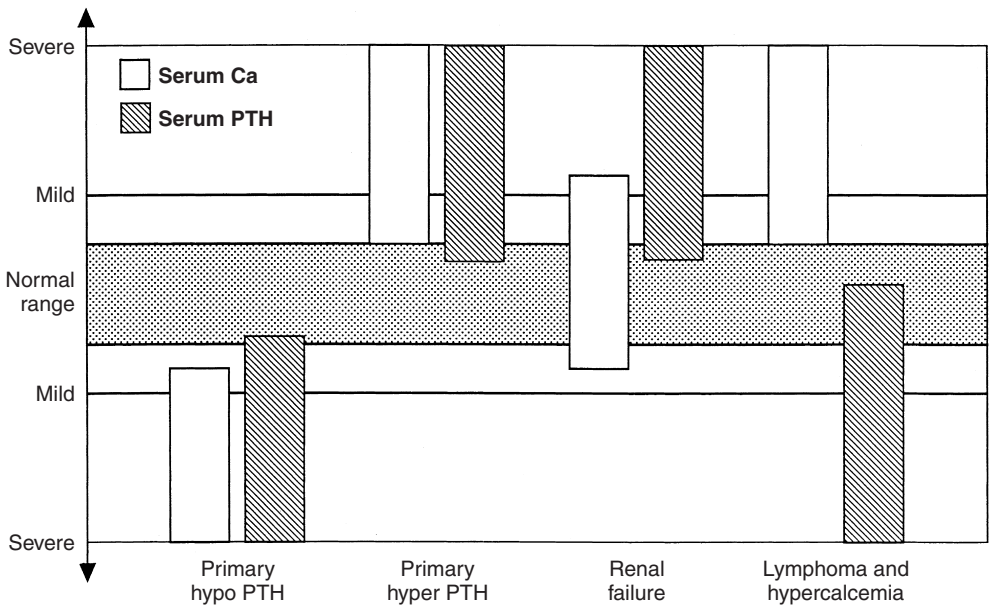

FIGURE 8-3. Range of serum calcium *(Ca)* and parathyroid hormone *(PTH)* concentrations for the more common disorders causing alterations in serum calcium or parathyroid gland function. (From Nelson RW, Couto CG: *Essentials of small animal internal medicine*, St Louis, 1992, Mosby.)

diet or after initiating prednisone treatment, but response is unpredictable.

Causes of Hypocalcemia • The most common causes of hypocalcemia in dogs and cats are puerperal tetany, acute and chronic renal failure, malassimilation syndromes, and primary hypoparathyroidism, (especially after thyroidectomy in hyperthyroid cats (Table 8-2). Hypocalcemia is present in hypoalbuminemic animals; however, the ionized fraction of calcium is assumed to be normal. The serum calcium concentration should be adjusted proportionate to a low serum total protein or albumin concentration before rendering a diagnosis of hypocalcemia. However, the association between total serum calcium concentration and serum albumin or protein concentration is weak, and serum ionized calcium concentrations can be decreased despite a "corrected" serum total calcium concentration that is in the normal range.

TABLE 8-2. Causes of Hypocalcemia in Dogs and Cats

Primary hypoparanthyroidism
Idiopathic
Post-thyroidectomy (bilateral)
Puerperal tetany (eclampsia)
Acute and chronic renal failure
Ethylene glycol toxicity
Acute pancreatitis
Intestinal malabsorption syndromes
Hypoproteinemia or hypoalbuminemia
Hypomagnesemia
Nutritional secondary hyperparathyroidism
Tumor lysis syndrome
Phosphate-containing enemas
Anticonvulsant medications
Sodium bicarbonate administration
Laboratory error

Hypocalcemia should be confirmed and total serum calcium concentration adjusted for low serum total protein or albumin concentration or serum ionized calcium concentration measured in the dog before initiating diagnostics. The list of differential diagnoses for hypocalcemia is relatively short, and the history, physical examination findings, CBC, serum biochemistry panel, and urinalysis usually provide the clues necessary to establish the diagnosis (Figure 8-4). Measurement of serum lipase concentration and serum trypsin-like immunoreactivity (TLI) and evaluation of an abdominal ultrasound should be done if pancreatitis is suspected (see Chapter 9). Primary hypoparathyroidism is the most likely diagnosis in the nonazotemic, nonlactating dog or cat with clinical signs of hypocalcemia. Documentation of a low baseline serum

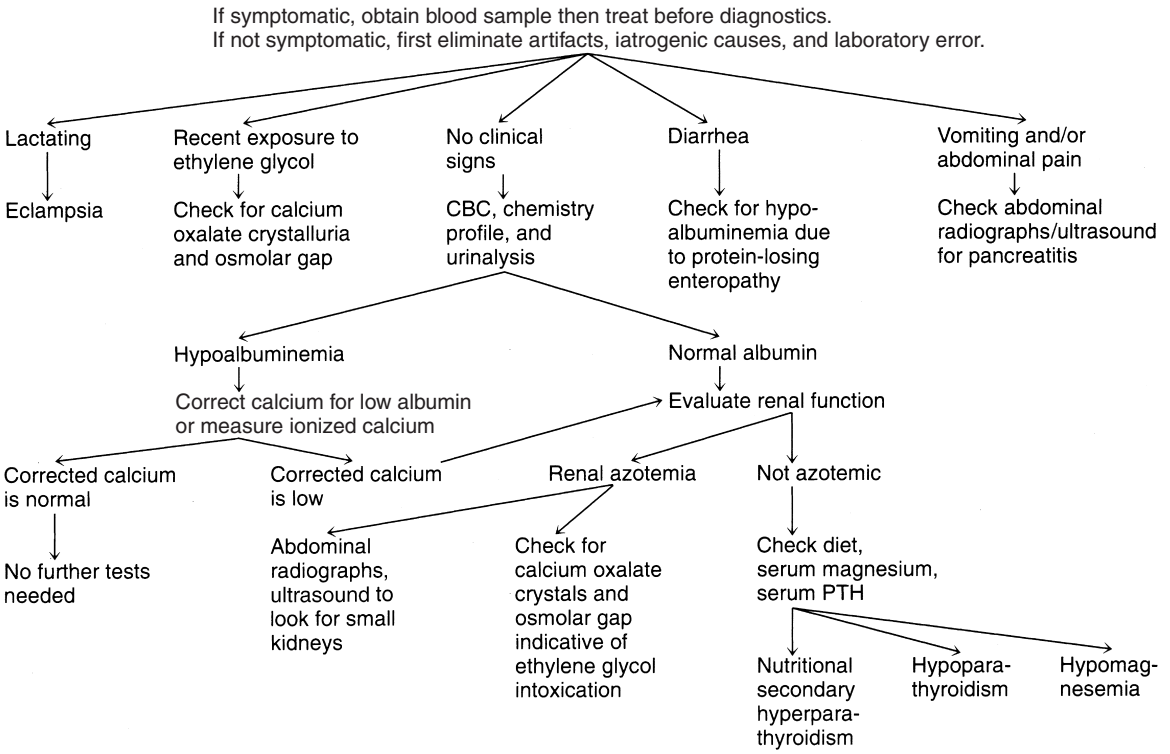

FIGURE 8-4. Diagnostic evaluation of hypocalcemia in dogs and cats. *CBC,* Complete blood count.

PTH concentration confirms this diagnosis (Figure 8-3) (Torrance and Nachreiner, 1989).

PHOSPHORUS

Commonly Indicated • Measurement of serum phosphorus concentration is commonly indicated in patients with any indications mentioned for calcium, plus unexplained hemolysis, seizures, or increased serum alkaline phosphatase (SAP).

Analysis • Phosphorus can be measured in serum, heparinized plasma, and urine as inorganic phosphorus by spectrophotometric methods.

Normal Values • Adult: 3.0 to 6.0 mg/dl.

Immature: Young dogs (i.e., < 12 months) especially of the large and giant breeds, and young cats (i.e., < 6 months) have higher serum phosphorus concentrations (dog, 4 to 9 mg/dl; cat, 4 to 8 mg/dl) than adults. Serum phosphorus concentration should decrease to adult values by 12 months of age.

To convert from mg/dl to mmol/L, multiply by 0.323.

Danger Values • Less than 1.5 mg/dl (hemolysis, neurologic signs).

Artifacts • Falsely increased: postprandial-protein intake (mild change), hemolysis, hyperlipidemia, hyperproteinemia, thrombocytosis. Effect is dependent on methodology used to measure phosphorus. Falsely decreased: postprandial-carbohydrate intake (mild change). (See Introduction to Serum Chemistries.)

Drug Treatments That May Alter Phosphorus Values • Phosphate-binding antacids, anesthetic agents, anticonvulsants, bicarbonate, diuretics, insulin, parenteral glucose administration, hyperalimentation, mithramycin, and salicylates may cause hypophosphatemia. Hyperphosphatemia may be caused by phosphate-containing enemas in cats or obstipated small dogs, IV potassium phosphate supplementation, anabolic steroids, vitamin D supplements, furosemide,

hydrochlorothiazide, and minocycline. Tetracyclines have a variable effect on phosphorus.

Causes of Hyperphosphatemia • Hyperphosphatemia can result from increased intestinal phosphate absorption, decreased phosphate excretion in urine, or a shift in phosphate from the intracellular to extracellular compartment. Translocation of phosphate between the intracellular and extracellular compartments is similar to that of potassium. The most common cause for hyperphosphatemia is decreased renal excretion secondary to renal failure (Table 8-3). History, physical examination, and routine clinical pathologic assessment (e.g., CBC, serum biochemical panel, urinalysis) usually enable the clinician to identify the cause. Azotemic patients may require additional tests to distinguish between prerenal, renal, and postrenal azotemia (see Chapter 7). (Evaluation for primary hypoparathyroidism is discussed under Causes of Hypocalcemia.) Serum thyroxine concentration should be determined in the nonazotemic cat with signs of hyperthyroidism (i.e., weight loss, polyphagia, restlessness). Survey skeletal radiographs may identify osseous neoplasia.

Causes of Hypophosphatemia • Hypophosphatemia results from decreased phosphate absorption in the intestinal tract, increased urinary phosphate excretion, or a shift from the extracellular to intracellular compartment. Hypophosphatemia is commonly associated with humoral hypercalcemia of malignancy (i.e., lymphosarcoma), primary hyperparathyroidism, and aggressive therapy for diabetic ketoacidosis (DKA) (see Table 8-3). Translocation of phosphate between the intracellular and extracellular compartments is similar to that of potassium. Factors that promote a shift of potassium into the intracellular compartment (e.g., alkalosis, insulin, glucose infusion) also promote a similar shift in phosphate.

When evaluating hypophosphatemia, one should eliminate artifacts and iatrogenic causes first. Mild hypophosphatemia (i.e., >2.0 mg/dl) without hypercalcemia is often ignored unless the animal is ketoacidotic (see Causes of Hyperglycemia). If concurrent hypercalcemia is present, diagnostic evaluation for hypercalcemia of malignancy and primary hyperparathyroidism should be performed (see Causes of Hypercalcemia).

TABLE 8-3. Causes of Altered Serum Phosphorus in Dogs and Cats

HYPERPHOSPHATEMIA	HYPOPHOSPHATEMIA
Young growing animal*	Decreased intestinal absorption
Renal failure*	Decreased dietary intake
Prerenal and postrenal azotemia*	Malabsorption, steatorrhea
Endocrine	Vomiting, diarrhea
Primary hypoparathyroidism	Phosphate-binding antacids*
Nutritional secondary hyperparathyroidism	Vitamin D deficiency
Hyperthyroidism (cats)	Increased urinary excretion
Acromegaly	Primary hyperparathyroidism*
Hypervitaminosis D	Hypercalcemia of malignancy*
Excess supplementation	Diabetic ketoacidosis
Cholecalciferol rodenticides*	Eclampsia
Jasmine toxicity	Hyperadrenocorticism
Osteolytic bone lesions (neoplasia)	Fanconi's syndrome (renal tubular defects)
Rhabdomyolysis	Diuretic administration
Trauma	Hypothermia recovery
Necrosis	Hyperaldosteronism
Tumor cell lysis syndrome	Aggressive parenteral fluid administration
Metabolic acidosis	Transcellular shift
Hemolysis	Insulin administration*
Drug—*see text*	Bicarbonate administration*
Iatrogenic	Parenteral glucose administration*
Intravenous phosphorus supplementation	Hyperalimentation
Phosphate-containing enemas	Respiratory, metabolic acidosis
Laboratory error	

*Common cause.

MAGNESIUM

Occasionally Indicated • Serum magnesium should be measured in dogs and cats with disorders and predisposing factors associated with hypomagnesemia and hypermagnesemia (Table 8-4), especially those with unexplained hypocalcemia (hypomagnesemia may inhibit the secretion and actions of PTH and promote calcium uptake into bone), hypokalemia resistant to parenteral supplementation (hypomagnesemia may cause potassium-losing nephropathy), DKA (hypomagnesemia may develop during the initial 24 hours of therapy), cardiac arrhythmias refractory to conventional therapy, and unexplained muscle weakness (including dysphagia and dyspnea), muscle fasciculations, or seizures.

Analysis • Magnesium concentration can be measured in serum or urine by spectrophotometric methods. Measurement of serum ionized magnesium concentration via an ion selective electrode more accurately assesses total body magnesium. Alternative methods of evaluating magnesium status include determining ultrafilterable magnesium and mononuclear blood cell magnesium content.

TABLE 8-4. Causes of Altered Serum Magnesium in Dogs and Cats

Hypomagnesemia
Gastrointestinal
Inadequate intake
Chronic diarrhea and vomiting
Malabsorption syndromes
Acute pancreatitis
Cholestatic liver disease
Nasogastric suction
Renal
Glomerulonephritis
Acute tubular necrosis
Postobstructive diuresis
Drug-induced tubular injury (e.g., aminoglycosides, cisplatin)
Prolonged intravenous fluid therapy
Diuretics
Digitalis administration
Hypercalcemia
Hypokalemia
Endocrine
Diabetic ketoacidosis
Hyperthyroidism
Primary hyperparathyroidism
Primary hyperaldosteronism
Miscellaneous
Acute administration of insulin, glucose, amino acids
Sepsis
Hypothermia
Massive blood transfusion
Peritoneal dialysis, hemodialysis
Total parenteral nutition
Hypermagnesemia
Renal insufficiency, failure
Excessive oral intake (e.g., antacids, laxatives)
Excessive parenteral administration (e.g., Mg^{+2}-containing fluids)

Normal Values • 1.5 to 2.5 mg/dl.

Danger Values • Development of clinical signs of hypomagnesemia varies widely, but treatment is probably indicated if serum magnesium concentration is less than 1.0 mg/dl. Serum magnesium concentrations greater than 10 mg/dl are associated with respiratory depression, apnea, coma, and cardiac arrest in people.

Artifacts • See Introduction to Serum Chemistries.

Drug Therapy That May Alter Serum Magnesium Concentration • Drug-induced renal tubular injury (e.g., cisplatin, aminoglycosides, amphotericin-B), diuretics (e.g., furosemide, thiazides), digitalis, insulin, glucose, amino acids, massive blood transfusion, and total parenteral nutrition solutions may cause hypomagnesemia. Magnesium-containing drugs (especially oral antacids and laxatives), chronic aspirin therapy, lithium, and progestagens may cause hypermagnesemia.

Causes of Hypomagnesemia • Hypomagnesemia results from decreased oral intake or gastrointestinal absorption of magnesium (e.g., small intestinal disease causing malabsorption), increased gastrointestinal loss (e.g., protracted vomiting, diarrhea), increased urinary magnesium excretion (e.g., interstitial nephritis, diuretics), or a shift of the cation from the extracellular to intracellular compartment. The most common causes of clinically significant hypomagnesemia include disorders causing small intestinal malassimilation, renal disorders with high urine output, osmotic diuresis of DKA, and shift of potassium, phosphate, and magnesium from the extracellular to intracellular compartment occurring within the first 24 hours of therapy for DKA (see Table 8-4). Magnesium is predominately an intracellular cation. Translocation of magnesium between the intracellular and extracellular compartments is similar to that of potassium. Factors that promote a shift of

potassium into the intracellular compartment (e.g., alkalosis, insulin, glucose infusion) also promote a similar shift of magnesium. During therapy, serum magnesium concentration can severely decline (i.e., < 1 mg/dl) because of the dilutional effects of fluid therapy and intracellular shift of magnesium after initiation of insulin and bicarbonate therapy.

Identifying hypomagnesemia is problematic because no simple, rapid, and accurate laboratory test is available to identify total body magnesium status. Approximately 1% of total body magnesium is present in serum; therefore, serum magnesium concentrations do not necessarily reflect total body magnesium status. A normal serum magnesium concentration can occur despite total body magnesium deficiency. A low serum magnesium concentration, however, supports a total body magnesium deficiency. Magnesium exists in three distinct forms in serum: (1) an ionized fraction, (2) an anion-complexed fraction, and (3) a protein-bound fraction. A serum ionized magnesium concentration determined using an ion-selective electrode more accurately assesses total body magnesium content than measurement of serum total magnesium and is recommended (Norris, Nelson, and Christopher, 1999). In animals with low serum magnesium concentration, a review of history, physical examination, and routine clinical pathologic assessment (e.g., CBC, serum biochemistry panel, urinalysis) usually provides clues to the underlying cause (see Table 8-4).

Causes of Hypermagnesemia • Hypermagnesemia occurs in animals with renal insufficiency (see Table 8-4) or is iatrogenically induced after excessive magnesium intake (e.g., IV administration, antacids, laxatives). Because the healthy kidney rapidly excretes excess magnesium, iatrogenically induced hypermagnesemia usually reflects underlying renal insufficiency. Hypermagnesemia has also been reported in cats with thoracic neoplasia and pleural effusion, although the mechanism involved with the development of hypermagnesemia in these cats is unknown (Toll et al, 2002). Measurement of serum magnesium concentration identifies hypermagnesemia. Evaluation of history, physical examination, and routine clinical pathologic assessments (e.g., CBC, serum biochemistry panel, urinalysis) usually identifies the cause of hypermagnesemia.

PARATHYROID HORMONE

Occasionally Indicated • Serum PTH concentration is required to diagnose primary hyperparathyroidism and hypoparathyroidism in animals with hypercalcemia and hypocalcemia, respectively.

Advantages • Serum PTH measurement can establish a diagnosis of primary parathyroid disease without surgical intervention.

Disadvantages • Validated PTH assays are limited, samples must be frozen during transit, and azotemia interferes with interpretation.

Analysis • PTH concentration is measured in serum by radioimmunoassay (RIA). The sample should be centrifuged as soon as possible after clotting, frozen, and shipped frozen to the laboratory. Different PTH assays measure different parts of the PTH molecule and may give different results in the same patient. The "two-site" PTH assay system uses two different polyclonal antibodies to measure midregion and C-terminal 39 to 84 amino acids and N-terminal 1 to 34 amino acids of PTH simultaneously, is valid for measurement of dog and cat PTH, and is currently used by most veterinary laboratories.

Normal Values • 2 to 13 pmol/L. Normal values may differ depending on the laboratory used.

Danger Values • None.

Artifacts • Prolonged storage or transport at temperatures above freezing may produce erroneous results.

Drug Treatment That May Alter PTH Concentrations • Any drug therapy affecting serum calcium concentration can affect serum PTH concentration (see Drug Therapy That May Alter Serum Calcium). Drugs that decrease serum calcium may increase serum PTH concentration and vice versa.

Causes of Increased Serum PTH Concentration • Disorders that cause increased serum PTH concentration include primary hyperparathyroidism, secondary renal hyperparathyroidism, secondary nutritional hyperparathyroidism, and nonparathyroid causes of hypocalcemia (see Causes of Hypocalcemia). A midnormal or increased serum PTH concentration in a hypercalcemic

patient with normal renal function strongly suggests primary hyperparathyroidism (see Figure 8-3) (Kallet et al, 1991; Torrance and Nachreiner, 1989). Animals with nonparathyroid-induced hypercalcemia have low to undetectable serum PTH concentrations. Serum PTH concentrations can be increased in animals with renal failure because of concurrent secondary renal hyperparathyroidism. Serum calcium concentration in these animals is usually in the normal range but may be decreased or, less commonly, increased with chronic end-stage renal failure. Serum ionized calcium concentration is usually normal in dogs and cats with secondary renal hyperparathyroidism. Serum calcium is in the low-normal or low range in animals with secondary nutritional hyperparathyroidism.

Causes of Decreased Serum PTH Concentration • Nondetectable serum PTH concentration in a hypocalcemic animal strongly suggests primary hypoparathyroidism. Patients with nonparathyroid-induced hypocalcemia should have normal or increased serum PTH concentrations. Nonparathyroid disorders causing hypercalcemia (see Causes of Hypercalcemia) also have low to undetectable serum PTH concentrations. The notable exception is hypercalcemia of chronic renal failure.

GLUCOSE

Commonly Indicated • Measurement of serum glucose concentration is commonly indicated in patients with polyuria, polydipsia, weakness, coma, behavioral change, or seizures (partial or complete). It should also be determined in patients with known hepatic or adrenal insufficiency, severe sepsis, pancreatic neoplasia, or glucosuria and in patients receiving insulin or total parenteral nutrition.

Advantages • The test is easily performed and readily available.

Disadvantages • The test is insensitive (provides a single determination); therefore, it may not detect disorders causing clinically significant hypoglycemia in some patients. In addition, the clinician must rapidly separate serum from red blood cells (RBCs) to prevent artifacts.

Analysis • Glucose can be measured in whole blood, serum, or plasma (lithium heparin, sodium fluoride, or EDTA). The clinician can measure blood glucose in two main ways: (1) reagent strips (with or without a reflectance meter) and (2) standard laboratory methods. The latter usually entails spectrophotometric analysis using either *o*-toluidine or enzymes (i.e., hexokinase, glucose oxidase). Reagent strips that use whole blood are quick, simple, inexpensive, and readily available. Correlation between results obtained with reagent strips and reflectance meter versus standard laboratory methods varies widely.

Differences between these methods are more pronounced at higher glucose concentrations (i.e., > 300 mg/dl). Blood glucose concentrations measured by reflectance meters are usually lower than corresponding results with standard laboratory methods.

Normal Values • 70 to 110 mg/dl.

To convert from mg/dl to mmol/L, multiply by 0.056.

Danger Values • Less than 40 mg/dl (coma or seizures) or greater than 1000 mg/dl (hyperosmotic diabetes with central nervous system [CNS] dysfunction and possible coma).

Artifacts • See Introduction to Serum Chemistries.

Serum or plasma must be separated from RBCs and white blood cells (WBCs) within 30 minutes after collection to minimize consumption of glucose by cells. At 22° C, glucose concentration decreases approximately 10% every 30 to 60 minutes, and this may occur more rapidly if large concentrations of metabolically active cells (e.g., leukocytosis, leukemia) are present.

When reagent strips are used, extremely increased or decreased packed cell volumes (PCVs) may alter the measured value. Reagent strips are less accurate at high glucose concentrations (i.e., > 300 mg/dl). Inadequate coverage of the reagent pad with blood may cause falsely low values. Expired reagent strips, meters that have not been properly calibrated, excessive washing or drying of the reagent strip, and inaccurate timing may also cause erroneous results.

Drug Therapy That May Alter Blood Glucose Concentration • Hypoglycemia may be caused by insulin, antihistamines, beta-blockers (e.g., propranolol), sulfonylureas

(e.g., chlorpropamide), ethanol, and, in diabetics, salicylates and anabolic steroids. The causes of hyperglycemia (especially in prediabetic patients) are L-asparaginase, beta-adrenergic drugs, corticosteroids, diazoxide, furosemide, acetazolamide, thiazides, salicylates, phenothiazines, nitrofurantoin, heparin, glucagon, thyroxine, progestagens, and estrogens. Megestrol acetate may cause transient or persistent hyperglycemia in cats.

Causes of Hypoglycemia • Hypoglycemia is typically the result of excessive glucose use by normal (e.g., with hyperinsulinism) or neoplastic cells, impaired hepatic gluconeogenesis and glycogenolysis (e.g., hepatic insufficiency), a deficiency in diabetogenic hormones (e.g., hypocortisolism), inadequate dietary intake of glucose and other substrates required for hepatic gluconeogenesis (e.g., starvation in neonates), or a combination of these mechanisms (e.g., sepsis; Table 8-5). Iatrogenic hypoglycemia is a common problem with overzealous insulin administration to diabetics.

Hypoglycemia should always be confirmed before beginning diagnostics to identify the cause. Careful evaluation of history, physical findings, and routine clinical pathologic assessments (e.g., CBC, serum biochemical panel, urinalysis) usually provide clues to the underlying cause (Figure 8-5). Idiopathic hypoglycemia, starvation, liver insufficiency (i.e., portal shunt), or sepsis usually causes hypoglycemia in the puppy or kitten. In young adults, hepatic insufficiency, hypoadrenocorticism, or sepsis usually causes hypoglycemia. In older animals, hepatic insufficiency, beta cell neoplasia, extrapancreatic neoplasia, hypoadrenocorticism, and sepsis are the most common causes.

Hypoglycemia tends to be mild (i.e., > 45 mg/dl) and is often an incidental finding in animals with hypoadrenocorticism and hepatic insufficiency. Additional alterations in clinical pathologic assessments (e.g., hyponatremia

TABLE 8-5. Causes of Altered Blood Glucose in Dogs and Cats

HYPOGLYCEMIA	HYPERGLYCEMIA
Beta cell tumor (insulinoma)	Diabetes mellitus*
Extrapancreatic neoplasia	"Stress" (cat)*
Hepatocellular carcinoma, hepatoma	Postprandial
Leiomyosarcoma, leiomyoma	Hyperadrenocorticism*
Hemangiosarcoma	Acromegaly (cat)
Hepatic insufficiency*	Diestrus (bitch)
Portal caval shunts	Pheochromocytoma (dog)
Chronic fibrosis, cirrhosis	Pancreatitis
Sepsis*	Exocrine pancreatic neoplasia
Hypoadrenocorticism	Renal insufficiency
Hypopituitarism	Drug therapy*
Idiophathic hypoglycemia*	Glucocorticoids
Neonatal hypoglycemia	Progestagens
Juvenile hypoglycemia (esp. toy breeds)	Megestrol acetate
Hunting dog hypoglycemia	Thiazide diuretics
Renal failure	Parenteral nutrition
Exocrine pancreatic neoplasia	
Hepatic enzyme deficiencies	
von Gierke's disease (type I glycogen storage disease)	
Cori's disease (type III glycogen storage disease)	
Severe polycythemia	
Prolonged starvation	
Prolonged sample storage*	
Iatogenic*	
Insulin therapy	
Sulfonylurea therapy	
Ethanol	
Ethylene glycol	
Artifact	
Glucometers	
Laboratory error	

*Common cause.

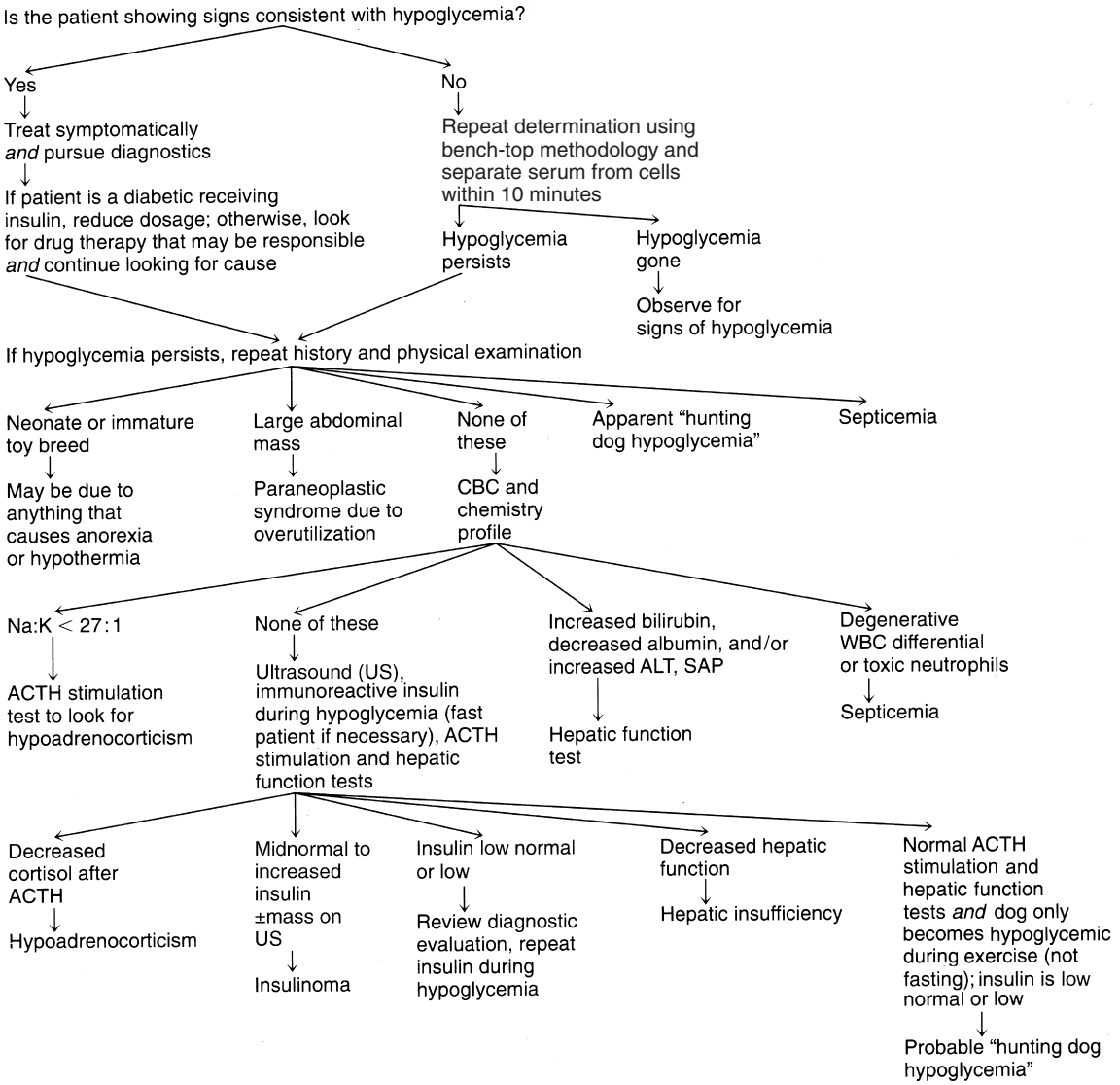

FIGURE 8-5. Diagnostic approach to hypoglycemia in dogs and cats. *ACTH*, Adrenocorticotropic hormone; *ALT*, alanine aminotransferase; *CBC*, complete blood count; *PTH*, parathyroid hormone; *SAP*, serum alkaline phosphatase; *WBC*, white blood cell.

or hyperkalemia [hypoadrenocorticism], increased alanine aminotransferase [ALT] activity, hypoalbuminemia [hepatic insufficiency]) are usually present. An adrenocorticotropic hormone (ACTH) stimulation test or hepatic function test (see Chapter 9) may be required to confirm the diagnosis. Severe hypoglycemia (i.e., < 35 mg/dl) may develop in neonates and juvenile kittens and puppies (especially toy breeds) and with sepsis, beta cell neoplasia, and extrapancreatic neoplasia (especially hepatic adenocarcinoma and leiomyosarcoma). Sepsis is readily identified by physical findings and CBC abnormalities, including a neutrophilic leukocytosis (typically > 30,000/µl), a shift toward immaturity, and toxic neutrophils (see Chapter 4). Extrapancreatic neoplasia can usually be identified on physical examination or on abdominal or thoracic imaging. Dogs with beta cell neoplasia typically have normal physical examination results and lack abnormalities except hypoglycemia. Measurement of baseline serum insulin concentration

when blood glucose is less than 60 mg/dl (preferably < 50 mg/dl) is necessary to confirm a beta cell tumor (see Insulin).

Causes of Hyperglycemia • Hyperglycemia results from insulin deficiency, impairment of insulin's action in peripheral tissues (i.e., decreased glucose use), increased hepatic gluconeogenesis and glycogenolysis, or a combination of these (see Table 8-5). Iatrogenic causes of hyperglycemia include IV infusion of dextrose-containing fluids and parenteral nutritional solutions, and administration of diabetogenic drugs (e.g., glucocorticoids, megestrol acetate). Infusion of fluids containing as little as 2.5% dextrose may cause hyperglycemia, depending on infusion rate and concurrent disorders interfering with carbohydrate tolerance. Severe hyperglycemia (typically without glucosuria) occurs commonly in "stressed" cats, presumably from epinephrine secretion. Many diseases also cause carbohydrate intolerance and mild hyperglycemia, primarily by interfering with insulin action in peripheral tissues.

Hyperglycemia between 130 and 180 mg/dl does not cause glucosuria, polyuria, or polydipsia. Hyperglycemia in this range is clinically silent and often an unsuspected finding. If the patient with mild hyperglycemia (i.e., < 180 mg/dl) also has polyuria-polydipsia, a disorder other than insulin-requiring diabetes mellitus should be sought. Mild hyperglycemia can occur up to 2 hours after eating in some animals after consumption of foods containing large amounts of monosaccharides and disaccharides, in stressed cats, with administration of diabetogenic medications, in early stages of diabetes mellitus, and with disorders decreasing insulin's effectiveness (see Table 8-5). A diagnostic evaluation for disorders causing insulin ineffectiveness is indicated if mild hyperglycemia (i.e., < 180 mg/dl) persists in the fasted, unstressed animal in which diabetogenic medications have been discontinued.

All hyperglycemic animals should be checked for glycosuria. Persistent hyperglycemia and glucosuria plus polyuria-polydipsia is diagnostic of diabetes mellitus. Persistence of diabetes mellitus depends, in part, on pathologic findings in the pancreatic islets, functional status of beta cells, and reversibility of concurrent diabetogenic diseases. Diabetogenic drugs (see Drug Therapy That May Alter Blood Glucose) should be stopped or adjusted; concurrent inflammatory, infectious, hormonal, or neoplastic disorders controlled or eliminated; and effects on blood glucose reevaluated in animals with suspected diabetes mellitus. Concurrent diabetogenic disorders are usually suggested from the history, physical examination, evaluation of routine clinical pathologic assessments (e.g., CBC, serum biochemical panel, urinalysis), and ease of glycemic regulation with insulin therapy. Concurrent diabetogenic disorders should always be suspected when glycemic control is difficult to attain with insulin therapy. The most common disorders interfering with glycemic regulation in dogs are obesity, chronic pancreatitis, hyperadrenocorticism, diestrus, and concurrent infection; in cats, the most common are obesity, chronic pancreatitis, hyperadrenocorticism, acromegaly, occult hyperthyroidism, and infection (Figure 8-6). Additional diagnostic tests (e.g., ACTH stimulation test, serum thyroxine concentration) may be necessary. Problems with insulin therapy itself (e.g., poor absorption, Somogyi phenomenon, short duration of insulin effect) should also be considered in the animal with poorly regulated diabetes. Assessment of insulin therapy by measuring blood glucose concentration every 1 to 2 hours is the first diagnostic step in identifying problems with insulin therapy.

INSULIN

Occasionally Indicated • Measurement of serum insulin concentration is performed to confirm an insulin-secreting beta cell tumor of the pancreas, to assess beta cell function in animals with diabetes mellitus, and to increase the clinician's index of suspicion for circulating insulin-binding antibodies in animals with diabetes mellitus and insulin resistance.

Advantages • Measurement of serum insulin concentration can establish a preoperative diagnosis of a beta cell tumor, which can be difficult to find at surgery.

Disadvantages • Many variables can affect serum insulin concentration. Interpretation must be done in conjunction with corresponding blood glucose concentration, and some RIAs for measuring insulin do not work in cats.

Analysis • Insulin is measured in serum by RIA. Insulin concentrations measured in

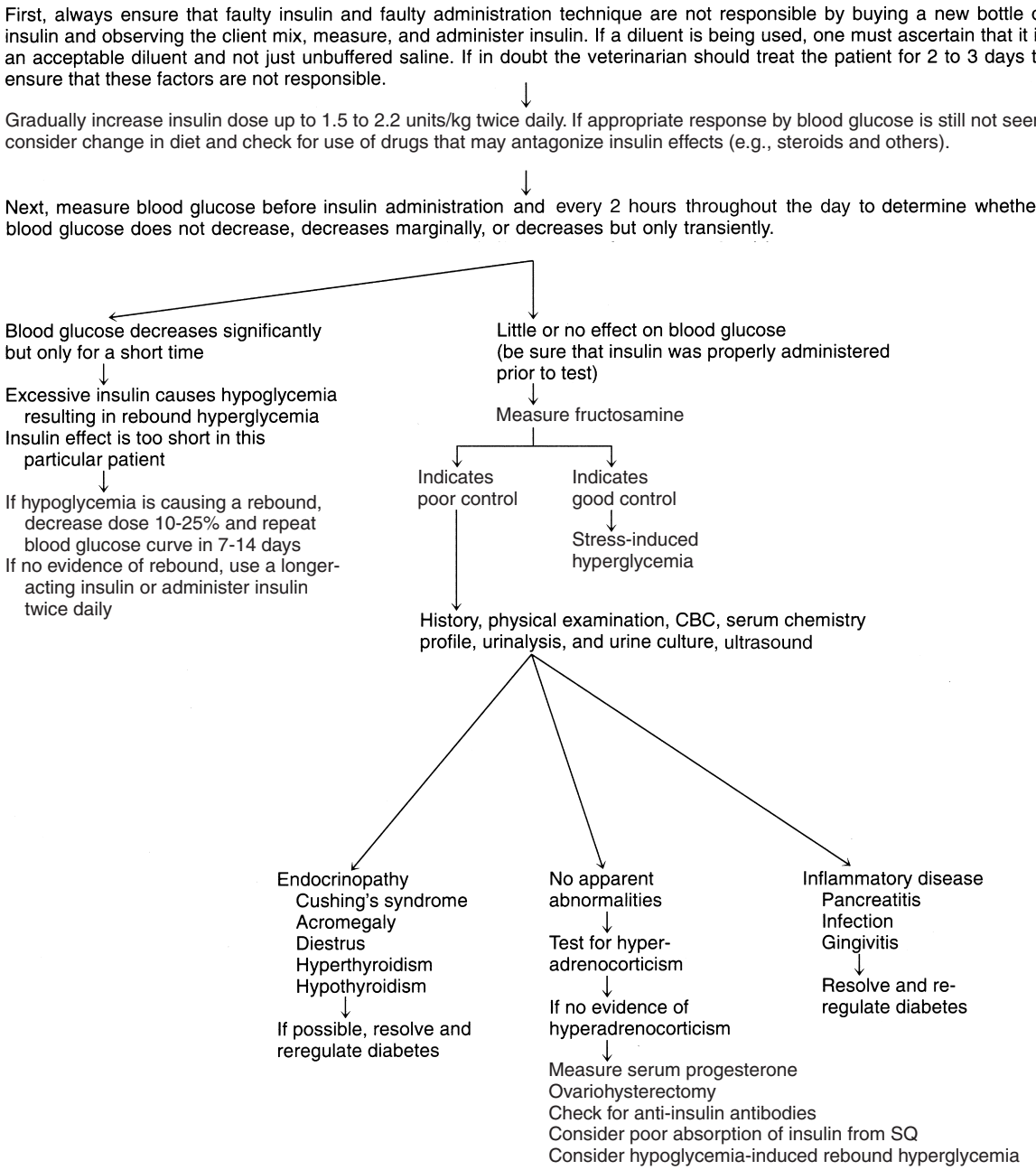

FIGURE 8-6. Diagnostic approach to persistent or inappropriate hyperglycemia in diabetics treated with insulin. *CBC,* Complete blood count; *SQ,* subcutaneous.

plasma tend to be higher than corresponding values in serum. Fasting samples are preferred to minimize the stimulatory effects of a meal on insulin secretion. Serum samples should be separated from cellular elements of blood and frozen before submission to the laboratory.

Normal Fasting Values • 5 to 20 μU/ml.

Fasting serum insulin concentration greater than 20 μU/ml in an untreated diabetic suggests type II diabetes or diabetes induced by concurrent insulin antagonistic disease.

To convert from μU/ml to pmol/L, multiply by 7.18.

Danger Values • None unless accompanied by hypoglycemia.

Artifacts and Effect of Drugs on Insulin • Serum insulin concentration is increased for several hours after a meal. In addition, many drugs and disorders affecting blood glucose concentration also affect serum insulin concentration. Insulin derived from an insulin injection may be measured in blood samples for up to 24 hours after the insulin injection. Chronic exogenous insulin therapy in diabetics may cause insulin antibody formation, which can interfere with single antibody RIA systems, causing spuriously increased values (i.e., >400 μU/ml).

Interpretation During Hypoglycemia • Confirmation of an insulin-secreting neoplasm requires documentation of inappropriate insulin secretion during hypoglycemia. If blood glucose concentration is less than 60 mg/dl (preferably <50 mg/dl) and serum insulin concentration is increased (i.e., >20 μU/ml), an insulin-secreting neoplasm is likely. If serum insulin is in the high-normal range (i.e., 10 to 20 μU/ml), an insulin-secreting tumor remains possible. Insulin values in the low-normal range (i.e., 5 to 10 μU/ml) may be found with other causes of hypoglycemia, as well as insulin-secreting tumors. Careful assessment of history, physical findings, clinical pathologic assessments, abdominal ultrasonography, and possibly repeated serum glucose and insulin values usually identifies the cause of hypoglycemia. A serum insulin concentration that is below normal range (i.e., <5 μU/ml) is inconsistent with an insulin-secreting tumor.

Interpretation During Hyperglycemia Serum insulin concentration should be increased (i.e., >20 μU/ml) during periods of hyperglycemia in normal animals. Documenting serum insulin concentration greater than 20 μU/ml in diabetics suggests residual beta cell function and either type II (i.e., noninsulin-dependent diabetes mellitus [NIDDM]) or diabetes induced by concurrent insulin antagonistic disease. Most animals with type I (i.e., insulin-dependent diabetes mellitus [IDDM]) have serum insulin concentrations less than 10 μU/ml. Markedly increased serum insulin concentration (i.e., >400 μU/ml) in blood obtained more than 24 hours after the last insulin injection in an insulin-treated diabetic suggests insulin-binding antibodies. Serum insulin concentration is typically less than 50 μU/ml 24 hours after the previous insulin injection in the diabetic without antibodies causing interference with the RIA.

INSULIN SECRETAGOGUE TESTING

Rarely Indicated • Insulin secretagogue testing can aid in differentiating type I (i.e., IDDM) from type II (i.e., NIDDM) diabetes mellitus, help identify carbohydrate intolerance in animals with suggested preclinical diabetes mellitus, and identify occult insulin-secreting tumors. The glucagon stimulation test can identify hyperadrenocorticism, portosystemic shunts, and hepatic glycogen storage diseases. The most common insulin secretagogue tests are the IV glucose tolerance test (IVGTT) and the IV glucagon stimulation test (IVGST).

Advantages • None.

Disadvantages • The tests are labor-intensive, expensive, often ineffective in differentiating type I from type II diabetes mellitus, and not recommended for diagnosis of insulin-secreting tumor, hyperadrenocorticism, or portosystemic shunts. Intraindividual variability in results of the IVGTT can be high in some cats (Sparkes et al, 1996).

Protocol • The animal should be fasted overnight. For the IVGTT, 0.5 g dextrose/kg body weight (using 50% dextrose solution) is administered IV over 30 seconds and blood samples are obtained prior to 1, 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes after glucose administration. The IVGST is preferred if fasting hyperglycemia greater than 200 mg/dl is present. For the IVGST, 0.5 mg (cat) or 1.0 mg (dog) of glucagon per patient is given IV and blood samples obtained before, 1, 5, 10, 20, 30, 45, and 60 minutes after glucagon administration. For both tests, serum should be harvested from each blood sample and frozen until glucose and insulin can be assayed.

Interpretation • Serum insulin concentration increases rapidly after IV administration of glucose or glucagon in the normal animal and is within 1 standard deviation (SD) of reference baseline mean serum insulin concentration by 60 minutes (Kaneko et al, 1977; Kirk, Feldman, and Nelson, 1993). In a diabetic, elevated fasting serum insulin concentration

or any post-secretagogue insulin concentration greater than 1 SD above reference mean (typically > 15 μU/ml) suggests residual beta cell function and the possibility of NIDDM. An increase in serum insulin concentration after IV administration of glucose or glucagon supports NIDDM, but failure to find increased serum insulin concentration does not rule out NIDDM.

Classification of a diabetic as IDDM or NIDDM ultimately depends on response to treatment. For identification of carbohydrate intolerance, blood glucose concentration should return to normal by 60 minutes after IV glucose or glucagon administration in the healthy animal. Persistent hyperglycemia (i.e., > 130 mg/dl) beyond this time suggests carbohydrate intolerance and a possible prediabetic state or stress-induced hyperglycemia (especially in cats). If desired, the glucose disappearance coefficient (K value) can be determined from the IVGTT using the following formula:

$$K = (0.693/t_{1/2}) \times 100$$

where $t_{1/2}$ is the halftime for glucose disappearance from serum (Kaneko et al, 1977). Linear regression analysis of a semilogarithmic plot of glucose concentration versus time is used to calculate the $t_{1/2}$, which is graphically estimated between 15 and 45 minutes after glucose injection. Normal animals typically have K values greater than 1.5% to 2.0%/minute, whereas a K value of less than 1.0%/minute is consistent with carbohydrate intolerance.

Artifacts • Any drug mentioned under "Drug therapy that may alter blood glucose" may alter the IVGTT and IVGST. Stress-induced hyperglycemia and anesthetic agents may also produce glucose intolerance, important in cats because of difficulty in taking multiple blood samples unless an IV catheter is used.

Warning • Glucagon administered to patients with pheochromocytoma may cause significant hypertension, whereas patients with insulin-secreting tumors may become severely hypoglycemic.

FRUCTOSAMINE

Commonly Indicated • Measurement of serum fructosamine concentration is used to monitor glycemic control in diabetic dogs and cats and occasionally to document persistent hyperglycemia and possible diabetes mellitus in animals with conflicting clinical signs and results of blood and urine glucose tests.

Analysis • Fructosamine is measured in serum by automated colorimetric assay using nitroblue tetrazolium chloride reduction method. Serum should be kept frozen until assayed for fructosamine.

Normal Values • 225 to 375 μmol/L. Normal values may differ depending on the laboratory used.

Artifacts • Hypoalbuminemia (decreased), hyperlipidemia (mild decrease in dogs), azotemia (mild decrease in dogs), storage at room temperature (decreased).

Drug Therapy That May Alter Serum Fructosamine Concentration • Any drugs that cause a persistent and prolonged increase (e.g., glucocorticoids) or decrease (e.g., chlorpropamide) in blood glucose concentration can increase or decrease serum fructosamine concentrations, respectively.

Interpretation in Diabetic Dogs and Cats • Fructosamines result from an irreversible, nonenzymatic, insulin-independent binding of glucose to serum proteins. The extent of glycosylation of serum proteins is directly related to the blood glucose concentration; the higher the average blood glucose concentration during the preceding 2 to 3 weeks, the higher the serum fructosamine concentration and vice versa. Serum fructosamine concentration is not affected by acute increases in the blood glucose concentration, as occurs with stress or excitement-induced hyperglycemia. Serum fructosamine concentrations can be measured during the routine evaluation of glycemic control performed every 3 to 6 months to (1) clarify the effect of stress or excitement on blood glucose concentrations, (2) clarify discrepancies between the history and physical examination findings and the serial blood glucose concentrations, and (3) assess the effectiveness of changes in insulin therapy (Elliott et al, 1999).

The normal reference range for serum fructosamine is determined in healthy dogs and cats with persistently normal blood glucose concentrations. Interpretation of serum fructosamine in a diabetic dog or cat must take into

consideration the fact that hyperglycemia is common, even in well-controlled diabetic animals. Most owners are happy with their pet's response to insulin treatment if serum fructosamine concentrations can be kept between 350 and 450 μmol/L. Values greater than 500 μmol/L suggest inadequate control of the diabetic state, and values greater than 600 μmol/L indicate serious lack of glycemic control. Serum fructosamine concentrations in the lower half of the normal reference range (i.e., < 300 μmol/L) or below the normal reference range should raise concern for significant periods of hypoglycemia in the diabetic dog or cat or reversion to a noninsulin-requiring diabetic state. The Somogyi phenomenon should be suggested if clinical signs (i.e., polyuria, polydipsia, polyphagia, weight loss) are present in a diabetic dog or cat with a serum fructosamine concentration less than 400 μmol/L. Increased serum fructosamine concentrations (i.e., > 500 μmol/L) suggest poor control of glycemia and a need for insulin adjustments. However, increased serum fructosamine concentrations do not identify the underlying problem.

Evaluation of the change in serum fructosamine can be used to assess response to changes in insulin therapy in fractious or stressed diabetic dogs and cats where reliability of blood glucose results are questionable. Because serum proteins have a relatively short half-life, serum fructosamine concentration changes relatively quickly (i.e., 2 to 3 weeks) in response to a change in glycemic control. This short period for change in serum fructosamine concentration is advantageous for detecting improvement or deterioration of glycemic control quickly. As such, serum fructosamine concentrations can be measured before and 2 to 3 weeks after changing insulin therapy to assess the effectiveness of the change. If changes in insulin therapy are appropriate, a decrease in serum fructosamine concentration should occur. If the serum fructosamine concentration is the same or has increased, the change was ineffective in improving glycemic control, another change in therapy based on an educated guess should be done, and the serum fructosamine measured again 2 to 3 weeks later.

HYPERLIPIDEMIA

Hyperlipidemia is an increase in plasma concentrations of cholesterol, triglycerides, or both. Because lipids are insoluble in water, they must be transported in blood with plasma proteins. Polar lipids (i.e., fatty acids, phospholipids, free cholesterol) are bound to albumin, whereas nonpolar lipids (i.e., triglycerides, cholesterol esters) are combined with carrier proteins (i.e., apoproteins) to form soluble macromolecular complexes called *lipoproteins*. Four major classes of lipoproteins are recognized: (1) chylomicrons, (2) very-low–density lipoproteins (VLDLs), (3) low-density lipoproteins (LDLs), and (4) high-density lipoproteins (HDLs). Triglyceride-rich lipoproteins are chylomicrons and VLDLs; chylomicrons are derived from dietary fat, and VLDLs are synthesized continuously by the liver and contain endogenously derived triglycerides. LDLs and HDLs act predominantly as vehicles for cholesterol and cholesterol esters. LDLs are a degradation product of VLDL metabolism; HDLs are produced in the liver and are the primary lipoprotein in the dog and cat.

Hyperlipidemia is often initially recognized by the finding of gross lipemia (i.e., milky plasma or serum) in a blood sample. Postprandial hyperlipidemia is normal; however, hyperlipidemia in the fasted (i.e., > 12 hours) animal is abnormal. Clear plasma or serum does not rule out hyperlipidemia, because hypercholesterolemia in the absence of hypertriglyceridemia does not cause lipemia. Lipemia is visible when serum triglycerides are greater than 200 mg/dl. Lipemia implies hypertriglyceridemia and an increase in chylomicrons, VLDLs, or both. Hyperlipidemia is also diagnosed after measurement of serum cholesterol and triglyceride concentrations. As a general rule of thumb, hyperlipidemia should be suspected when serum cholesterol and triglyceride concentrations exceed 300 mg/dl and 150 mg/dl, respectively, in the fasted adult dog, and 200 mg/dl and 100 mg/dl, respectively, in the fasted adult cat.

Hyperlipidemia may be idiopathic, a primary defect in lipoprotein metabolism, or a consequence of systemic disease (Table 8-6). Postprandial hyperlipidemia is the most common cause of hyperlipidemia and should be eliminated before one performs more costly tests. The transient increase in serum triglycerides typically resolves within 10 hours after eating. Postprandial plasma cholesterol concentration usually does not exceed the upper limit of the species-specific reference range.

For most animals, persistent hyperlipidemia is caused by an endocrine or metabolic disorder. The patient may be presented

TABLE 8-6. Causes of Hyperlipidemia in Dogs and Cats

Postprandial hyperlipidemia*
Secondary hyperlipidemia*
Hypothyroidism
Diabetes mellitus
Hyperadrenocorticism
Pancreatitis
Cholestasis
Hepatic insufficiency
Nephrotic syndrome
Primary hyperlipidemia
Idiopathic hyperlipoproteinemia* (Miniature Schnauzers)
Idiopathic hyperchylomicronemia (cat)
Lipoprotein lipase deficiency (cat)
Idiopathic hypercholesterolemia
Drug-induced hyperlipidemia
Glucocorticoids
Megestrol acetate (cat).

*Common cause.

because of signs related to the underlying disorder (see Table 8-6) or signs caused by hyperlipidemia, or it may be asymptomatic. Persistent hyperlipidemia is an indication for further tests to eliminate potential secondary causes. History, physical examination, CBC, serum biochemistry panel, serum lipase (controversial; see Chapter 9), thyroxine concentration, and urinalysis make up the initial diagnostic approach to hyperlipidemia (Figure 8-7).

NOTE: Hypertriglyceridemia may falsely increase serum lipase concentration in dogs. Additional diagnostic tests (e.g., abdominal ultrasound, ACTH stimulation test, serum trypsin-like immunoreactivity) may be indicated, depending on initial test results.

Idiopathic hyperlipidemias include idiopathic hyperlipoproteinemia of miniature schnauzers, idiopathic hyperchylomicronemia, feline lipoprotein lipase deficiency, and idiopathic hypercholesterolemia. Idiopathic or primary hyperlipidemia is diagnosed after eliminating secondary causes of persistent hyperlipidemia. Idiopathic and primary hyperlipidemias can be further characterized by lipoprotein electrophoresis or combined ultracentrifugation and precipitation techniques. These techniques require specialized equipment and are not widely available commercially. An idea of the nature of lipoprotein

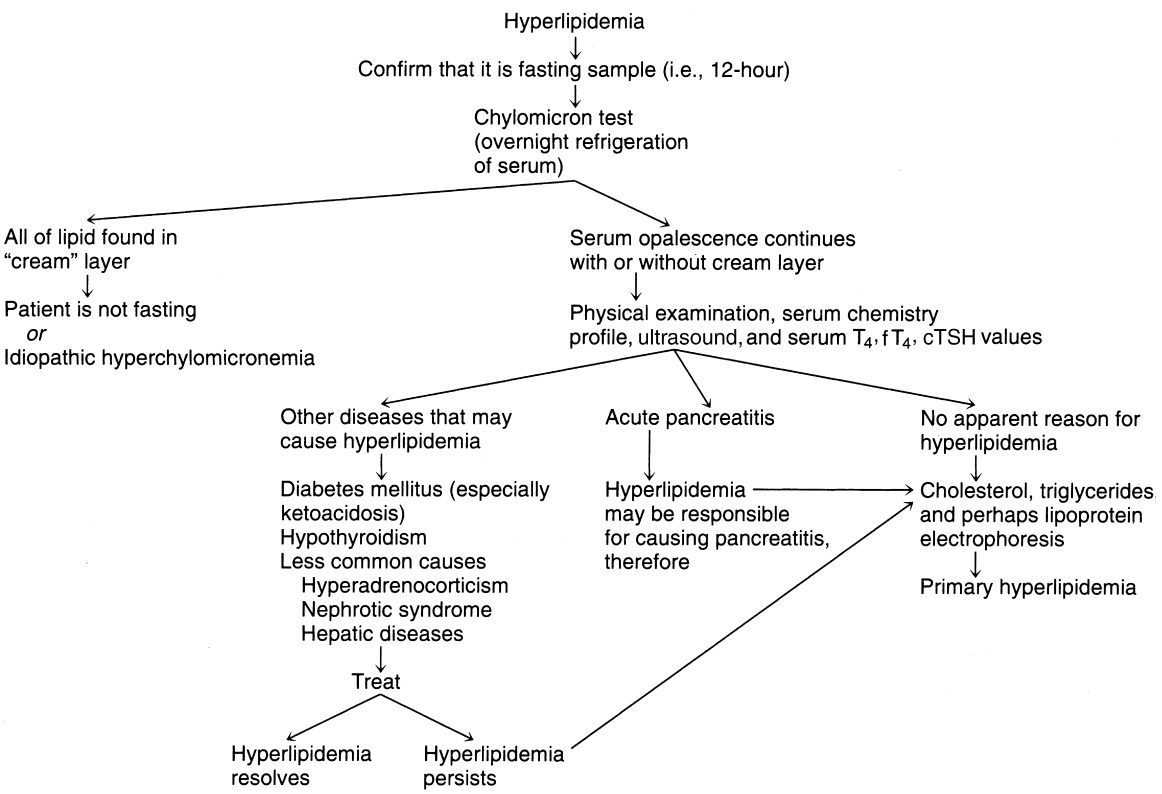

FIGURE 8-7. Diagnostic approach to hyperlipidemia in the dog and cat. *cTSH*, Canine thyroid-stimulating hormone; T_4, thyroxine; fT_4, free thyroxine.

alterations can also be gained by measuring serum cholesterol and triglyceride concentrations and performing the chylomicron test. In the chylomicron test, the lipemic plasma or serum sample is left at 4°C overnight (i.e., 12 hours) and then assessed visually. Chylomicrons form a cream layer at the top of the sample, whereas persistent lactescence of plasma or serum is caused by VLDLs.

CHOLESTEROL

Occasionally Indicated • Measurement of serum cholesterol concentration is indicated in dogs and cats with hyperlipidemia and as a screening test for hypothyroidism and hyperadrenocorticism. Hypercholesterolemia by itself does not cause lipemia.

Analysis • Cholesterol is measured in serum or heparinized plasma by spectrophotometric, chromatographic, automated direct, and enzymatic methods. Automated direct techniques may slightly overestimate serum cholesterol concentration.

Normal Values • Dogs, 125 to 300 mg/dl; cats, 75 to 200 mg/dl. To convert from mg/dl to mmol/L, multiply by 0.026.

Danger Values • None.

Artifacts • See Introduction to Serum Chemistries.

Drug Therapy That May Alter Serum Cholesterol Concentration • L-asparaginase, azathioprine, colchicine, cholestyramine, and oral aminoglycosides may cause hypocholesterolemia. Corticosteroids, methimazole, phenytoin, prochlorperazine, thiazides, and phenothiazines may cause hypercholesterolemia.

Causes of Hypocholesterolemia • Rarely a problem, hypocholesterolemia primarily occurs with protein-losing enteropathy, hepatopathy (especially portal caval shunt and cirrhosis), selected malignancies, and severe malnutrition.

Causes of Hypercholesterolemia • Diet or spontaneous disease (see Table 8-6) may cause hypercholesterolemia. Feeding a very high fat diet or sampling blood shortly after eating may cause minor elevations in serum cholesterol concentration. The diagnostic approach to persistent hypercholesterolemia in the fasted animal is as outlined for hyperlipidemia (see Figure 8-7). Primary differentials are diabetes mellitus, hypothyroidism, hyperadrenocorticism, and protein-losing disorders, most notably involving the kidney.

TRIGLYCERIDES

Occasionally Indicated • Occasionally, measurement of serum triglyceride concentration is indicated in patients with hyperlipidemia or hypercholesterolemia, especially if clinical signs associated with hypertriglyceridemia are present (i.e., seizures, gastrointestinal signs, lipemia retinalis, xanthomata, peripheral neuropathy). Lipemia implies hypertriglyceridemia.

Analysis • Triglycerides are measured in serum or EDTA plasma by spectrophotometric or enzymatic methods. Triglyceride values tend to be slightly less in plasma than in serum. Enzymatic methods may give slightly greater values than spectrophotometric methods.

Normal Values • Dogs, 10 to 150 mg/dl; cats, 5 to 100 mg/dl.

Danger Values • Greater than 1000 mg/dl (neurologic signs, seizures).

Artifacts • Lipemia may interfere with several routine biochemistry tests (Table 8-7), depending upon the species evaluated (i.e., dog versus cat), analytic instrumentation, laboratory methods, and severity of hypertriglyceridemia. See Introduction to Serum Chemistries.

Drug Therapy That May Alter Serum Triglyceride Concentration • Ascorbic acid, L-asparaginase, and heparin may cause hypotriglyceridemia. Different anabolic steroids have different effects. Estrogens and cholestyramine may cause hypertriglyceridemia.

Causes of Hypotriglyceridemia • Hypotriglyceridemia is not clearly associated with any disease; it rarely occurs in hyperthyroidism (?) and some malabsorptive protein-losing enteropathies (?).

Causes of Hypertriglyceridemia • Hypertriglyceridemia may be idiopathic,

TABLE 8-7. Effect of Lipemia on Clinical Chemistry Analytes* in Canine and Feline Sera

FALSE INCREASE IN VALUES		FALSE DECREASE IN VALUES	
CANINE SERA	**FELINE SERA**	**CANINE SERA**	**FALINE SERA**
Total bilirubin	Total bilirubin	Creatinine	Creatinine
Phosphorous	Phosphorous	Total CO_2	Total CO_2
Alkaline phosphate (SAP)†	Alkaline phosphate (SAP)†	Cholestrol	ALT
Glucose†	Glucose†	Urea nitrogen	
Total protein‡	Total protein‡		
Lipase			
Alanine aminotransferase (ALT)			

*Analytes were measured using Coulter DACES (Coulter Diagnostics, Hialeah, FL).
†Interference only occurs at very high concentrations of lipid.
‡When measured using refractometer.
Adapted from Jacobs RM et al: Effects of bilirubinemia, hemolysis, and lipemia on clinical chemistry analytes in bovine, canine, equine, and feline sera. *Can Vet J* 1992; 33:605-608.

develop as a primary defect in lipoprotein metabolism, or be a consequence of an underlying systemic disease (see Table 8-6). Postprandial hypertriglyceridemia is the most common cause of hyperlipidemia and should be eliminated before more costly tests are performed (see Hyperlipidemia). The diagnostic approach for persistent hypertriglyceridemia in the fasted animal is as outlined for hyperlipidemia (see Figure 8-7). Primary differentials are diabetes mellitus, hypothyroidism, acute pancreatitis, and idiopathic hyperlipoproteinemia.

LIPOPROTEIN ELECTROPHORESIS

Rarely Indicated • Lipoprotein electrophoresis is rarely needed in patients with unexplained, persistent, fasting hyperlipidemia.

Disadvantages • Lipoprotein electrophoresis requires specialized equipment. Lack of diagnostic specificity is a problem. Because of differences in dogs and cats versus humans, a combination of laboratory methods may be necessary to specifically evaluate a given lipoprotein class and prevent erroneous interpretations of results.

Analysis • The test is performed on serum or EDTA plasma.

Normal Values • Dogs (see Zerbe, 1986); cats (see Bauer, 1992).

Danger Values • None.

Artifacts • Grossly lipemic serum or plasma should not be allowed to separate and have the "cream layer" (i.e., chylomicrons) discarded before testing.

Drug Treatment That May Alter Results • See Cholesterol and Triglycerides.

Causes of Altered Patterns • Although lipoprotein electrophoresis allows the diagnostician to determine if chylomicrons, VLDLs, LDLs, HDLs, or a combination thereof is present, it seldom allows identification of a specific disease.

THYROXINE

Commonly Indicated • Measurement of serum thyroxine (T_4) concentration is performed to diagnose hypothyroidism and hyperthyroidism and to monitor sodium levothyroxine therapy for hypothyroidism and methimazole therapy for hyperthyroidism. Clinical signs suggestive of hypothyroidism include mental dullness, lethargy, exercise intolerance or unwillingness to exercise, gain in weight without a corresponding increase in appetite or food intake, endocrine alopecia, seborrhea, pyoderma, weakness, neurologic signs (e.g., facial nerve paralysis, head tilt, ataxia, seizures), failure to cycle (bitch), and failure to grow (i.e., cretinism). Clinical signs suggestive of hyperthyroidism include weight loss (which may progress to cachexia), polyphagia, restlessness or hyperactivity, haircoat changes (e.g., patchy alopecia, matted hair, lack of or excessive grooming behavior),

polyuria, polydipsia, vomiting, diarrhea, and aggressive behavior. Hyperthyroidism should also be considered in an older cat with a palpable cervical nodule.

Advantages • Tests to measure serum T_4 are readily available; T_4 is a stable hormone and the primary hormone secreted by the thyroid gland.

Disadvantages • A myriad of variables can affect serum T_4 concentration, resulting in misinterpretation.

Analysis • T_4 concentration is measured in serum by RIA or in-house ELISA. T_4 concentration in serum is stable for at least 8 days at room temperature. Hemolysis, freezing, and thawing does not affect serum T_4 concentrations. Despite stability, serum samples should be frozen and sent to the laboratory on cool packs. The laboratory must use an RIA validated for the species being tested. When evaluating sodium levothyroxine therapy, serum should be obtained 4 to 6 hours after sodium levothyroxine administration for dogs on twice-a-day therapy and before and 4 to 6 hours after sodium levothyroxine administration for dogs on once-a-day therapy (see Chapter 18). A single serum sample can be obtained at any time when evaluating methimazole therapy in cats.

Normal Values • Dogs, 1.0 to 3.5 µg/dl; cats, 1.0 to 4.0 µg/dl.

NOTE: Different laboratories have different normal ranges. To convert from µg/dl to nmol/L, multiply by 12.87.

Interpretation for hypothyroidism in dogs:
Greater than 2.0 µg/dl: hypothyroidism very unlikely
1.5 to 2.0 µg/dl, hypothyroidism unlikely
1.0 to 1.5 µg/dl, unknown
0.5 to 1.0 µg/dl, hypothyroidism possible
Less than 0.5 µg/dl, hypothyroidism very likely

Interpretation for hyperthyroidism in cats:
Greater than 4.0 µg/dl, hyperthyroidism very likely
3.0 to 4.0 µg/dl, hyperthyroidism possible
2.5 to 3.0 µg/dl, unknown
2.0 to 2.5 µg/dl, hyperthyroidism unlikely
Less than 2.0 µg/dl, hyperthyroidism very unlikely

NOTE: The clinician must always consider clinical signs, physical findings, clinical pathologic changes, effects of concurrent drugs and illness, and index of suspicion when interpreting serum T_4 results.

Danger Values • None.

Variables That Affect Serum T4 Concentration • Variables affecting serum T_4 concentration can be divided into physiologic, pharmacologic, and systemic illness (Table 8-8). Serum T_4 concentration is higher in young dogs (i.e., < 1 year of age) and decreases with advancing age (Reimers et al, 1990). Smaller breeds have higher T_4 concentration than large or giant breeds. Certain breeds (e.g., sight hounds) have lower serum T_4 values than other breeds. Estrus, pregnancy, and obesity increase serum T_4 concentration, whereas hypoproteinemia may decrease serum T_4 concentration. Antithyroid hormone antibodies may develop in dogs with lymphocytic thyroiditis and cause spuriously increased or decreased serum T_4 values (Thacker, Refsal, and Bull, 1992). The effect of antithyroid hormone antibodies on the serum T_4 value depends on the type of assay being used by the laboratory. Drugs that have been documented to alter serum T_4 concentration in dogs are listed in Table 8-8; the most clinically relevant is concurrent glucocorticoid administration. Many nonthyroidal illnesses are associated with decreased serum T_4 concentration (Table 8-9). This phenomenon is called the *euthyroid sick syndrome*. The severity of illness has a direct correlation with the severity of suppression of serum T_4 concentration.

Causes of Decreased Serum T4 Concentration • The primary disorder causing decreased serum T_4 concentration is hypothyroidism, which must be differentiated from all the variables listed earlier that can also suppress serum T_4 concentration (Table 8-10). In the dog, lymphocytic thyroiditis, idiopathic atrophy, or neoplastic destruction of the thyroid gland may cause primary hypothyroidism. Secondary hypothyroidism results from a deficiency of pituitary gland thyrotropin (thyroid-stimulating hormone [TSH]) and may be caused by pituitary malformation, destruction, or suppression (e.g., glucocorticoid therapy). Feline hypothyroidism is usually iatrogenic, resulting from bilateral thyroidectomy, excessive methimazole or,

TABLE 8-8. Variables That May Affect Baseline Serum Thyroid Hormone Function Test Results in Dogs

Age	Inversely proportional effect
Neonate (<3 mo)	Increased T_4
Aged (>6 yr)	Decreased T_4
Body size	Inversely proportional effect
Small (<10 kg)	Increased T_4
Large (>30 kg)	Decreased T_4
Breed	
Sight hounds (e.g., Greyhound)	T_4 and free T_4 lower than normal range established for dogs; no difference for TSH
Gender	No effect
Time of day	No effect
Weight gain/obesity	Increased
Weight loss/fasting	Decreased T_4, no effect on free T_4
Strenuous exercise	Increased T_4, decreased TSH, no effect on free T_4
Estrus (estrogen)	No effect on T_4
Pregnancy (progesterone)	Increased T_4
Surgery/anesthesia	Decreased T_4
Concurrent illness*	Decrease T_4 and free T_4; depending on illness, TSH may increase, decrease or not change
Drugs	
Carprofen	Decreased T_4, free T_4 and TSH
Etodolac	No effect on T_4, free T_4 or TSH
Glucocorticoids	Decreased T_4 and free T_4; decrease or no effect on TSH
Furosemide	Decreased T_4
Methimazole	Decreased T_4 and free T_4; increased TSH
Phenobarbital	Decreased T_4 and free T_4; delayed increase in TSH
Phenylbutazone	Decreased T_4
Potassium bromide	No effect on T_4, free T_4 or TSH
Progestagens	Decreased T_4
Propylthiouracil	Decreased T_4 and free T_4; increased TSH
Sulfonamides	Decreased T_4 and free T_4; increased TSH
Ipodate	Increased T_4, decreased T_3
Dietary iodine intake	If excessive, decreased T_4 and free T_4; increased TSH
Thyroid hormone autoantibodies	Increased or decreased T_4; no effect on free T_4 or TSH

*There is a direct correlation between severity and systemic nature of illness and suppression of serum T_4 and free T_4 concentration.
From Nelson RW, Couto GC: *Small animal internal medicine,* ed 3, St Louis, 2003, Mosby.

rarely, radioactive iodine (^{131}I) therapy for hyperthyroidism. Naturally occurring feline hypothyroidism is rare and is usually identified in kittens. Congenital hypothyroidism results in a cretin.

Diagnosis of hypothyroidism must consider history, physical findings, clinical pathologic assessments, serum T_4 concentration, and the clinician's index of suspicion for the disease (Table 8-11). Fasting lipemia, hypercholesterolemia and, less commonly, mild normocytic normochromic anemia (i.e., hematocrit 30% to 35%) are the most common abnormalities identified on routine clinical pathologic assessments. A mild-to-moderate increase in lactate dehydrogenase, aspartate aminotransferase, alanine transaminase, alkaline phosphatase and, rarely, creatine kinase activity may also occur. These increased activities are extremely inconsistent, however, and may not be directly related to the hypothyroid state. Mild hypercalcemia may occur in some dogs with congenital hypothyroidism, and skeletal survey radiographs may identify delayed epiphyseal ossification, epiphyseal dysgenesis (i.e., irregularly formed, fragmented, or stippled epiphyseal centers), short broad skulls, shortened vertebral bodies, and decreased length of diaphyses of long bones (Saunders and Jezyk, 1991). Additional diagnostic tests, including baseline serum free thyroxine (fT_4) and endogenous TSH concentration, TSH or thyroid-releasing hormone (TRH) stimulation test, or clinical response to trial therapy with sodium levothyroxine, are usually required to establish the diagnosis.

Monitoring serum concentrations during levothyroxine therapy allows the clinician to evaluate the dose, frequency of administration, and adequacy of intestinal absorption of sodium levothyroxine (see Chapter 18). With appropriate supplementation, all serum T_4 concentrations should be 1.5 to 5.0 μg/dl. Serum TSH concentrations should also be in the normal range. Serum T_4 concentration less than 1.5 μg/dl, especially less than 1.0 μg/dl,

TABLE 8-9. Common Causes of the "Euthyroid Sick Syndrome"

Acute diseases
Bacterial bronchopneumonia
Sepsis
Distemper
Autoimmune hemolytic anemia
Systemic lupus erythematosus
Intervertebral disk disease
Polyradiculoneuritis
Acute renal failure
Acute hepatitis
Acute pancreatitis
Chronic diseases
Generalized demodicosis
Generalized bacterial furunculosis
Systemic mycoses
Lymphosarcoma
Chronic renal failure
Diabetes mellitus
Congestive heart failure
Cardiomyopathy
Chronic hepatitis, cirrhosis
Obesity
Gastrointestinal disturbances
Megaesophagus

Data from Feldman EC, Nelson RW: Hypothyroidism. In *Canine and feline endocrinology and reproduction*, Philadelphia, 1996, WB Saunders.

suggests inadequate thyroid hormone supplementation, especially if the serum TSH concentration is increased and clinical signs of hypothyroidism persist. Finding increased postpill serum T_4 concentrations is not an absolute indication to reduce dose of sodium levothyroxine, especially if no clinical signs of thyrotoxicosis are observed. However, a reduction in dose is recommended whenever serum T_4 concentrations are greater than 7.5 μg/dl.

Causes of Increased Serum T_4 Concentration • The primary disorder causing increased serum T_4 concentration is hyperthyroidism (see Table 8-10). Spontaneous feline hyperthyroidism is usually caused by multinodular adenomatous goiter. Less common are thyroid adenomas that cause enlarged, distorted lobes; malignant thyroid carcinoma accounts for less than 5% of clinical cases. Functional thyroid tumors are uncommon in dogs but, when present, may be adenoma or carcinoma.

TABLE 8-10. Causes of Altered Serum T_3 and T_4 Values in Dogs and Cats

Decreased serum thyroid hormone values
Hypothyroidism (primary and secondary)
Nonthyroidal illness (euthyroid sick syndrome) (see Table 8-9)
Drugs (see Table 8-8)
Iatrogenic
Post-thyroidectomy (bilateral)
Increased serum thyroid hormone values
Hyperthyroidism
Drugs (see Table 8-8)
Thyroid hormone autoantibodies
Normal dog or cat
Iatrogenic
Excess thyroid hormone supplementation

Oversupplementation of sodium levothyroxine to dogs with hypothyroidism and spurious increase because of antithyroid hormone antibody interference with RIAs for serum T_4 measurement can also cause increased serum T_4 concentrations. Oversupplementation is diagnosed by history; antithyroid hormone antibodies should be suspected in dogs with clinical signs of hypothyroidism but increased serum T_4 concentration. Serum T_4 concentration above the normal range also occurs in normal dogs; 2% to 3% of the normal population of dogs falls outside two SDs from the mean used to establish the normal range. Evaluation of serum TSH is useful in differentiating the causes of increased serum T_4 concentration in dogs.

History and physical examination are the basis for suspecting hyperthyroidism. Results of a CBC are usually normal in hyperthyroid cats. The most common abnormalities are a mild increase in the PCV and mean corpuscular volume (MCV). Common serum biochemical abnormalities include an increase in serum activities of ALT, alkaline phosphatase, and aspartate aminotransferase; the increase is typically in the mild-to-moderate range (i.e., 100 to 400 IU/L). One or more of these hepatic enzymes is increased in approximately 90% of hyperthyroid cats. Additional evaluation of the liver should be considered if hepatic enzyme activities are greater than 500 IU/L. Increased serum urea nitrogen and creatinine concentrations are identified in approximately 30% and hyperphosphatemia in 20% of hyperthyroid cats, findings that have important implications from the standpoint of the initial treatment implemented. The urine specific gravity ranges from 1.008 to greater than 1.050. Most hyperthyroid cats have urine specific gravities greater than 1.035. The documentation of a concentrated urine specific gravity is helpful in differentiating primary renal insufficiency from prerenal azotemia in cats with an increased blood urea nitrogen (BUN). ECG abnormalities include tachycardia, increased R wave amplitude in lead II and, less commonly, right bundle

TABLE 8-11. Diagnostic Recommendations for Evaluating Thyroid Gland Function in the Dog

1. The decision to assess thyroid gland function should be based on results of the initial evaluation, which includes history, physical examination, and results of routine bloodwork (i.e., complete blood count, serum biochemistry panel, urinalysis).
2. The initial screening tests for thyroid gland function in *pet dogs* are the components in statement 1 and baseline serum T_4 concentration.
   a. Treatment is indicated if serum T_4 concentration is low and initial evaluation of the dog strongly supports the diagnosis of hypothyroidism.
   b. Treatment is not indicated if serum T_4 is normal and initial evaluation of the dog does not support the diagnosis of hypothyroidism.
   c. Additional diagnostic tests (i.e., endogenous thyroid-stimulating hormone [cTSH] and free T_4 by equilibrium dialysis) are indicated if serum T_4 concentration is normal but initial evaluation of the dog strongly supports the diagnosis of hypothyroidism *or* the veterinarian is uncertain if hypothyroidism exists after evaluation of history, physical examination, routine bloodwork, and baseline serum T_4 concentration.
3. The initial screening tests for thyroid gland function in *potential breeding or show dogs with clinical signs* are the components in statement 1 and baseline serum free T_4 concentration measured by equilibrium dialysis, cTSH concentration, and thyroglobulin autoantibody test. Measurement of baseline serum T_4 concentration is optional.
   a. Treatment is indicated if all of these test results are abnormal and initial evaluation of the dog strongly supports the diagnosis of hypothyroidism.
   b. Treatment is not indicated if all of these tests are normal and initial evaluation of the dog does not support the diagnosis of hypothyroidism.
   c. Treatment is not indicated and tests should be repeated in 2 to 4 weeks if the dog has clinical signs suggestive of hypothyroidism, all tests are normal or only one test is abnormal and another cause for the clinical signs cannot be identified.
   d. If two tests are abnormal, especially free T_4 and cTSH concentration, the decision to treat or wait and repeat the tests in 2 to 4 weeks should be based on results of clinical evaluation of the dog and index of suspicion for the disease.
4. The recommended tests to screen the thyroid gland in the *healthy breeding dog* are the components in statement 1 and baseline serum free T_4 concentration measured by equilibrium dialysis, cTSH concentration, and thyroglobulin autoantibody test.
   a. The thyroid gland is normal and the dog considered safe to breed if it has no clinical signs and physical examination, results of routine bloodwork, and all thyroid gland tests are normal. Normal results do not rule out possible development of hypothyroidism in the future. Annual evaluation of the thyroid gland should be performed in potential breeding dogs, beginning at 1 year of age.
   b. If any thyroid gland test is abnormal, we are uncertain of the importance or significance of such results. One may wish to not use the dog for breeding and have the thyroid gland tests repeated in 1 to 6 months. As long as any of the thyroid gland tests remains abnormal, future development of hypothyroidism should be considered possible and breeding is not recommended.

branch block, left anterior fascicular block, widened QRS complexes, and atrial and ventricular arrhythmias. Thoracic radiographs may reveal cardiomegaly, pulmonary edema, or pleural effusion. ECG abnormalities depend on the form of thyrotoxic cardiomyopathy. The reader is referred to a text on ultrasonography for details of echocardiographic abnormalities.

Measurement of baseline serum T_4 concentration is reliable in diagnosing hyperthyroidism in most animals (Figure 8-8). Cats with mild or occult hyperthyroidism and hyperthyroid cats with significant nonthyroidal illness (e.g., neoplasia, systemic infection, organ system failure) can have "normal" serum T_4 concentrations. If the serum T_4 test result is not definitive, the recommendation is to measure serum T_4 and free T_4 (fT_4) using the modified equilibrium dialysis technique in 1 to 2 weeks and to rule out nonthyroidal illness. If the diagnosis is still not established, the veterinarian should consider repeating the serum T_4 and fT_4 tests in 4 to 8 weeks, obtaining a radionuclide thyroid scan, or completing a T_3 suppression test. If available, a radionuclide thyroid scan is preferable over the T_3 suppression test for diagnosing hyperthyroidism in cats with nondiagnostic serum T_4 and fT_4 concentrations.

3,5,3′-TRIIODOTHYRONINE

Indications • Measurement of serum 3,5,3′-triiodothyronine (T_3) concentration is done during the T_3 suppression test for hyperthyroidism in cats. Although theoretic indications for the measurement of serum T_3 concentration are the same as that for serum T_4, baseline serum T_3 concentration offers little additional diagnostic information beyond that obtained with serum T_4 concentration in identifying feline hyperthyroidism. It offers minimal to no value in assessing canine thyroid gland

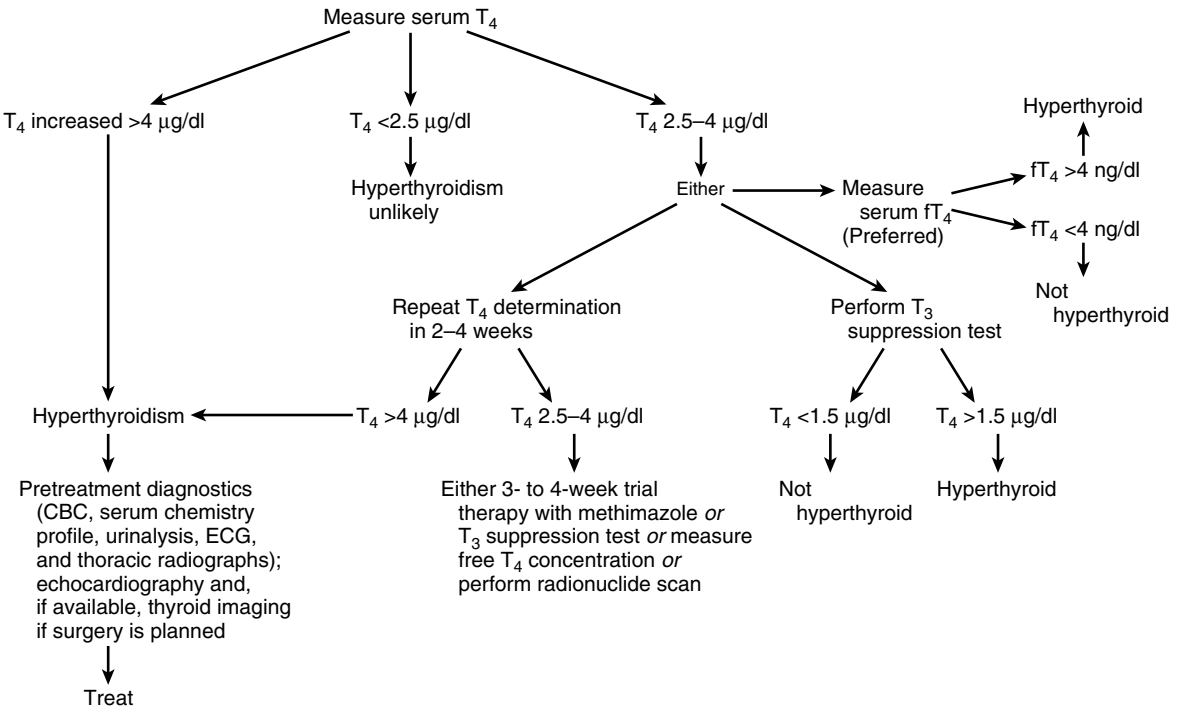

FIGURE 8-8. Evaluation of cats with suspected hyperthyroidism based on history, clinical signs (i.e., weight loss, polyphagia, polyuria-polydipsia, hyperactivity, cervical mass) or laboratory findings (i.e., increased serum alkaline phosphatase [SAP], increased serum phosphorus). *CBC*, Complete blood count; *ECG*, electrocardiogram; *T_4*, thyroxine; *fT_4*, free thyroxine; *T_3*, triiodothyronine.

function (Nelson et al, 1991). Measurement of serum T_3 concentration is not currently recommended for assessment of thyroid gland function.

Advantages • None.

Disadvantages • Measurement of serum T_3 concentration is of questionable diagnostic usefulness; a myriad of variables can affect serum T_3 concentration.

Analysis • Same as for serum T_4.

Normal Values • Dogs, 0.5 to 1.8 ng/ml; cats, 0.4 to 1.6 ng/ml.

NOTE: Different laboratories have different normal ranges.

Danger Values • None.

Variables That Affect Serum T_3 Concentration • The variables are the same as for serum T_4 concentration. The incidence of anti-T_3 antibodies is greater than anti-T_4 antibodies in dogs with lymphocytic thyroiditis.

Causes of Decreased Serum T_3 Concentration • The causes are the same as for serum T_4 concentration.

Causes of Increased Serum T_3 Concentration • Same as for serum T_4 concentration. Spurious increase as the result of antithyroid hormone antibody interference with single antibody RIAs is more common with T_3 than T_4.

FREE THYROXINE

Indications • fT_4 concentration is measured to assess thyroid gland function in dogs with suspected hypothyroidism and cats with occult hyperthyroidism. Measurement of baseline serum fT_4 concentration by modified equilibrium dialysis provides a more consistent and reliable assessment of thyroid gland function than measurement of serum total T_4 concentration.

Advantages • The test is readily available and provides a more accurate assessment of thyroid gland function than serum T_4 concentration

when measured by modified equilibrium dialysis (MED) techniques. The hormone is stable during handling and shipping.

Disadvantages • Variables may affect serum fT_4 concentration, resulting in misinterpretation of results. In addition, the test is relatively expensive.

Analysis • Serum fT_4 is currently measured using two methods: (1) RIA using kits designed for use in human beings and (2) a MED technique that uses a short dialysis step to separate fT_4 from protein-bound T_4 followed by RIA for fT_4. The MED technique is the most accurate method for determining serum fT_4 concentrations and is the preferred fT_4 test for assessing thyroid gland function in dogs. Accuracy of the MED assay has been greater than 90% in all studies in which it has been critically evaluated, compared with an accuracy of 75% to 85% for serum T_4 (Peterson, Melian, and Nichols, 1997).

Normal Values • Dogs, 0.8 to 3.5 ng/dl; cats, 1.0 to 4.0 ng/dl.

NOTE: Different laboratories have different normal ranges. To convert from ng/dl to pmol/l, multiply by 12.87.

Danger Values • None.

Variables That Affect Serum fT_4 Concentration • Concurrent illness can suppress serum fT_4 concentrations, although alterations in serum concentrations of fT_4 are more variable than with serum T_4 and probably depend in part on the pathophysiologic mechanisms involved in the illness. In general, serum fT_4 concentrations tend to be decreased in dogs with concurrent illness but to a lesser extent than total T_4 concentrations. However, fT_4 concentrations can be less than 0.5 ng/dl if severe illness is present. Drugs that suppress serum T4 can also suppress serum fT_4 concentrations (see Table 8-8). Unlike serum T_4, circulating antithyroid hormone antibodies do not affect the fT_4 results determined by the MED test. The impact of many of the other variables known to affect serum T_4 concentrations have not yet been reported for serum fT_4. Until clinical studies indicate otherwise, the clinician should assume that variables affecting serum T_4 may have a similar affect on serum fT_4 concentration.

Causes of Decreased Serum fT_4 Concentration • Causes are the same as for serum T_4 concentration. In the authors' laboratory, serum fT_4 values less than 0.8 ng/dl (especially < 0.5 ng/dl) suggest hypothyroidism assuming that history, physical examination, and clinicopathologic abnormalities are also consistent with the disorder, and severe concurrent illness is not present.

Causes of Increased Serum fT_4 Concentration • Causes are the same as for serum T_4 concentration. Measurement of serum fT_4 concentration by the MED technique is a more reliable means of assessing thyroid gland function than measurement of the serum total T_4 concentration in cats with hyperthyroidism. In part, this is because nonthyroidal illness has more of a suppressive effect on serum total T_4 than fT_4, and serum fT_4 is increased in many cats with occult hyperthyroidism and "normal" T_4 test results (Peterson, Melian, and Nichols, 2001). Because of cost, measurement of fT_4 concentration by MED is often reserved for cats with suspected hyperthyroidism where T_4 values are borderline. Occasionally, concurrent illness causes an increase in serum fT_4 concentration in cats (an increase that can exceed the reference range). For this reason, serum fT_4 concentration should always be interpreted in conjunction with total T_4 concentration measured from the same blood sample. An increased serum fT_4 concentration in conjunction with high-normal or increased serum T_4 concentration is supportive of hyperthyroidism. An increased serum fT_4 concentration in conjunction with a low-normal or low serum T_4 concentration is supportive of euthyroid sick syndrome rather than hyperthyroidism.

BASELINE ENDOGENOUS CANINE THYROID-STIMULATING HORMONE

Indications • Baseline serum canine TSH (cTSH) is measured to assess thyroid gland function in dogs with suspected hypothyroidism. Theoretically, measurement of cTSH in conjunction with serum T_4 concentration, fT_4 concentration, or both should increase accuracy of diagnosing dogs with suspected hypothyroidism.

Advantages • The test is readily available, the hormone is stable during handling and shipping, and testing may improve accuracy

of assessing thyroid gland function when used in conjunction with measurement of other thyroid hormones.

Disadvantages • The test is of minimal diagnostic usefulness by itself: normal cTSH concentrations are common in dogs with hypothyroidism, and increased cTSH values are common in dogs with euthyroid sick syndrome. Current cTSH assays cannot identify low cTSH values.

Analysis • Currently, two validated cTSH assays are available for dogs: (1) a commercial immunoradiometric assay and (2) an in-house cTSH assay offered by the Endocrine Section, Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University. These assays provide similar values for serum cTSH concentrations in healthy and hypothyroid dogs. Currently no validated assay is available for measuring endogenous TSH in cats.

Normal Values • Dogs, 0.01 to 0.6 ng/ml.

Danger Values • None.

Variables That Affect Serum cTSH Concentration • Many of the variables that decrease baseline thyroid hormone concentrations, most notably concurrent illness, may also increase serum TSH concentrations in euthyroid dogs, potentially causing misdiagnosis of hypothyroidism if the clinician accepts the results out of context (see Table 8-8). Serum TSH concentrations may be normal or increased depending, in part, on the effect of the concurrent illness on fT_4 concentrations and on pituitary function. If pituitary function is suppressed, cTSH concentrations will be in the normal range or undetectable. If pituitary response to changes in fT_4 concentration is not affected by the concurrent illness, cTSH concentrations will increase in response to a decrease in fT_4. Serum cTSH concentrations can easily exceed 1.0 ng/ml in dogs with the euthyroid sick syndrome.

Causes of Increased Serum cTSH Concentration • In dogs, increased serum cTSH concentration is caused by primary hypothyroidism and euthyroid sick syndrome. Ideally, baseline endogenous cTSH should be increased and serum T_4 and fT_4 decreased in dogs with primary hypothyroidism. Unfortunately, serum TSH concentrations measured in hypothyroid dogs overlap with those for euthyroid dogs with concurrent illness, and approximately 20% of hypothyroid dogs have normal TSH concentrations (i.e., <0.6 ng/ml). In most studies, the sensitivity and specificity of the TSH assay have averaged approximately 80% (Scott-Moncrieff et al, 1998). Serum TSH concentration should always be interpreted in conjunction with the serum T_4 or fT_4 concentrations measured in the same blood sample and should never be used as the sole test of thyroid gland function. Finding a low serum T_4 or fT_4 concentration and a high TSH concentration in a blood sample obtained from a dog with appropriate history and physical examination findings supports the diagnosis of primary hypothyroidism, and finding normal serum T_4, fT_4, and TSH concentrations rules out hypothyroidism. Any other combination of serum T_4, fT_4, and cTSH concentrations is difficult to interpret; however, based on the accuracy of the assays, reliance on fT_4 measured by MED technique is recommended (see Table 8-11). A normal serum T_4 or fT_4 concentration and an increased serum TSH concentration are found in the early stages of primary hypothyroidism in humans. Although similar thyroid hormone and TSH results have been identified in dogs, it is not known what percent of these dogs will progress to clinical hypothyroidism. Clinical signs of hypothyroidism are usually not evident in these dogs, in part because serum T_4 and fT_4 concentrations are in the normal range. Treatment with sodium levothyroxine is not indicated. Instead, assessment of thyroid gland function should be repeated in 2 to 4 months, especially if antibody tests for lymphocytic thyroiditis are positive. If progressive destruction of the thyroid gland is occurring, serum T_4 and fT_4 will gradually decrease, serum TSH increase with time, and clinical signs will eventually develop.

THYROID HORMONE AUTOANTIBODIES

Indications • Measurement of thyroid hormone autoantibodies is done to explain unusual serum T_4 or T_3 values in dogs with suspected hypothyroidism, to identify lymphocytic thyroiditis in dogs with hypothyroidism, and possibly as a screening test for lymphocytic thyroiditis in currently euthyroid dogs intended for breeding (see Thyroglobulin Autoantibodies). Circulating thyroid hormone antibodies may interfere with RIA techniques used to measure serum T_4 and T_3 concentrations,

causing spurious, unreliable numbers (Thacker, Refsal, and Bull, 1992; Young, Sartin, and Kemppainen, 1985). The type of interference depends on the RIA. Falsely low results are obtained with nonspecific separation methods (e.g., ammonium sulfate, activated charcoal), whereas falsely increased values occur with single antibody systems using antibody-coated tubes. Determination of accurate T_4 or T_3 concentration in these patients requires special extraction techniques or removal of the endogenous immunoglobulin before assay. Fortunately, the prevalence of clinically relevant concentrations of thyroid hormone antibody causing obvious spurious T_4 values accounts for less than 1% of assayed blood samples. Serum fT_4 measured by MED is not affected by T_4 autoantibodies and should be evaluated in lieu of serum T_4 in dogs suspected of having T_4 autoantibodies.

See Appendix I for availability of measurement of circulating antibodies directed against T_3 and T_4. A positive thyroid hormone antibody titer in a dog with appropriate clinical signs, clinical pathologic assessments, and low or high serum thyroid hormone concentration supports hypothyroidism caused by lymphocytic thyroiditis. Interpretation of a positive thyroid hormone antibody titer in an asymptomatic dog with no clinical pathologic abnormalities and normal serum thyroid hormone concentrations is uncertain. This may be an early marker for lymphocytic thyroiditis that progresses to symptomatic hypothyroidism, or it may be an incidental or transient finding. The percentage of euthyroid dogs positive for thyroid hormone autoantibodies eventually developing overt hypothyroidism is unknown (see discussion of Thyroglobulin Autoantibodies).

THYROGLOBULIN AUTOANTIBODIES

Indications • Measurement of thyroglobulin (Tg) autoantibodies is done to identify lymphocytic thyroiditis in dogs with hypothyroidism and possibly as a screening test for lymphocytic thyroiditis in currently euthyroid dogs intended for breeding. Tg autoantibodies occur in conjunction with T_3 and T_4 autoantibodies and are identified in dogs with lymphocytic thyroiditis that are not positive for T_3 and T_4 autoantibodies, implying that Tg autoantibody determination is a better screening test for lymphocytic thyroiditis than T_3 and T_4 autoantibodies. Presence of serum Tg autoantibodies implies thyroid pathology but provides no information on the severity or progressive nature of the inflammatory response or the extent of thyroid gland involvement, nor is this test an indicator of thyroid gland function. Tg autoantibodies should not be used alone in the diagnosis of hypothyroidism. Dogs with confirmed hypothyroidism can be negative and euthyroid dogs can be positive for Tg autoantibodies. Identification of Tg autoantibodies would support hypothyroidism caused by lymphocytic thyroiditis if the dog has clinical signs, physical findings, and thyroid hormone test results consistent with the disorder.

Tg autoantibodies may be used as a prebreeding screen for lymphocytic thyroiditis in valuable breeding dogs. Currently, a positive Tg autoantibody test is considered suggestive of lymphocytic thyroiditis and supports retesting in several months before breeding the dog. The value of serum Tg autoantibodies as a marker for eventual development of hypothyroidism remains to be clarified. A recent 1-year prospective fT_4 study found that approximately 20% of 171 dogs with positive Tg autoantibody and normal fT_4 and cTSH test results developed changes in fT_4 or cTSH test results (or both) consistent with hypothyroidism. In addition, 15% reverted to a negative Tg autoantibody test with no change in fT_4 and cTSH test results, and 65% remained Tg autoantibody positive or had an inconclusive result with no change in fT_4 and cTSH test results 1 year later (Graham et al, 2001).

3,5,3′-TRIIODOTHYRONINE SUPPRESSION TEST

Indications • A T_3 suppression test is done to confirm hyperthyroidism in the cat with occult disease. The T_3 suppression test evaluates responsiveness of pituitary TSH secretion to suppression by sodium liothyronine. Administration of T_3 to normal cats should suppress pituitary TSH secretion, decreasing the serum T_4 concentration. Subsequent measurement of serum T_4 is accurate because exogenous T_3 cannot be converted to T_4. Cats with hyperthyroidism experience autonomous secretion of thyroid hormone. Administration of T_3 to hyperthyroid cats should have no suppressive effect.

Advantages • The test is readily available, relatively inexpensive, easy to interpret.

Disadvantages • It requires 3 days to complete, and accuracy depends on the owner's ability to administer the drug seven times and on the cat swallowing the tablets.

Protocol • Serum is obtained for determination of baseline serum T_4 and T_3 concentration. Owners then administer sodium liothyronine (synthetic T_3)* beginning the following morning at a dose of 25 μg three times a day for 2 days. On the morning of day 3, a seventh 25 μg dose is administered. Two to 4 hours later, a second blood sample is obtained for measurement of serum T_4 and T_3 concentrations.

Interpretation • Normal cats have postpill serum T_4 concentrations less than 1.5 μg/dl. Hyperthyroid cats have postpill T_4 concentrations greater than 2.0 μg/dl. Values between 1.5 and 2.0 μg/dl are nondiagnostic. The percentage of decrease in serum T_4 concentration is not reliable, although suppression greater than 50% below baseline occurs in normal but not hyperthyroid cats (Peterson, Graves, and Gamble, 1990).

Serum T_3 results are used to determine whether the owner successfully administered the thyroid medication to the cat. Serum T_3 concentration should increase in all cats properly tested, regardless of status of thyroid gland function. If serum T_4 concentration fails to decline in a cat in which serum T_3 concentration has not increased, problems with owner compliance are likely and test results are discarded.

THYROID-STIMULATING HORMONE STIMULATION TEST

Indication • A TSH stimulation test is done to confirm hypothyroidism in dogs. The primary advantage of this test is differentiation of hypothyroidism from the euthyroid sick syndrome in a dog with low baseline serum T_4 or fT_4 concentration.

Advantages • The test is easy to perform and usually accurate in differentiating hypothyroidism from other disorders decreasing serum T_4 concentrations.

Disadvantages • It is expensive, TSH is sporadically available, and results are uninterpretable in some dogs.

*Cytobin, SmithKline Beecham Animal Health, Exton, PA.

Protocol • A blood sample is obtained immediately before and 6 hours after IV administration of 0.1 unit TSH/kg (maximum, 5 IU TSH/dog). Serum T_4 is measured in each blood sample. The remaining reconstituted TSH can be refrigerated for 3 weeks or frozen for 3 months without loss of biologic activity.

Interpretation • Interpretation is based on absolute serum T_4 values. In the authors' laboratory and using the TSH protocol described earlier, euthyroid dogs have a post-TSH serum T_4 concentration greater than 3 μg/dl, whereas dogs with primary hypothyroidism have a post-TSH serum T_4 concentration below normal baseline serum T_4 range (i.e., < 1.5 μg/dl) (see Figure 8-9) (Nelson et al, 1991). Post-TSH serum T_4 concentrations of 1.5 to 3 μg/dl are nondiagnostic and may occur in early hypothyroidism or may represent thyroid gland suppression because of concurrent illness or drug therapy in an otherwise euthyroid dog. Severe systemic illness, however, can cause post-TSH serum T_4 concentrations considered diagnostic for primary hypothyroidism (i.e., < 1.5 μg/dl). Similarly, low baseline serum T_4 concentration with normal post-TSH serum T_4 concentration is also difficult to interpret. Assessment of history, physical findings, clinical pathologic assessment, and sometimes response to trial therapy may be required to ultimately determine thyroid gland function. Pre- and post-TSH serum T_4 concentrations above baseline in conjunction with a lack of response of the thyroid gland to TSH (see Figure 8-9), occur occasionally in dogs with hypothyroidism and may reflect circulating thyroid hormone antibodies in a dog with lymphocytic thyroiditis.

THYROID-RELEASING HORMONE STIMULATION TEST

Indications • A TRH stimulation test is done to confirm canine hypothyroidism. The primary advantage of this test is differentiation of hypothyroidism from the euthyroid sick syndrome in dogs with low baseline serum T_4 and fT_4 concentrations. The TRH stimulation test is no longer recommended for identification of occult hyperthyroidism in cats, in part because of adverse effects of TRH in cats and the existence of better tests to identify occult hyperthyroidism.

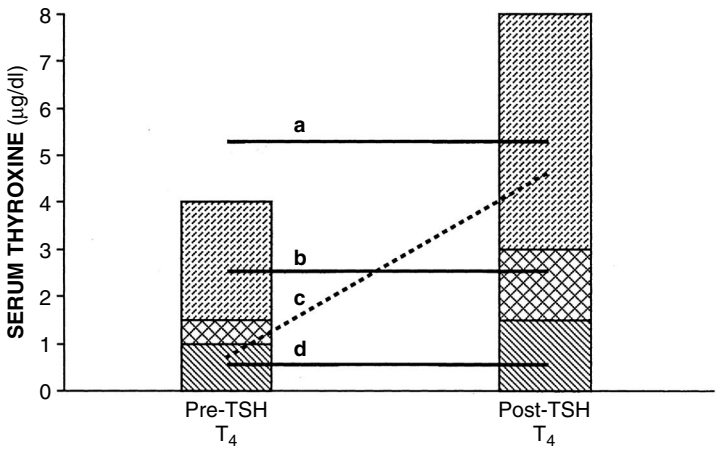

FIGURE 8-9. Interpretation of thyroid-stimulating hormone *(TSH)* stimulation test in dogs. Post-TSH thyroxine *(T_4)* values that fall into the normal range usually indicate normal thyroid gland function. Exceptions include high pre-TSH T_4 values with no increase in post-TSH T_4 *(line a)* and low pre-TSH T_4 values with normal response to TSH *(line c)*. Primary hypothyroidism with anti-T_4 antibodies *(lines a and b)* and secondary hypothyroidism or the suppressive effects of concurrent disease *(line c)* should be considered. Low pre-TSH and post-TSH T_4 values *(line d)* and post-TSH T_4 values in the nondiagnostic range may be indicative of hypothyroidism or the suppressive effects of concurrent disease. *Slanted dashes*, normal; *crosshatches*, nondiagnostic range; *slanted lines*, hypothyroid range. (From Nelson RW, Couto CG: *Essentials of small animal internal medicine*, St Louis, 1992, Mosby-Year Book, p 549.)

Advantages • The test is easy to perform, and TRH is readily available.

Disadvantages • It is expensive, uninterpretable results are more common than in TSH stimulation tests in dogs, and adverse reactions are frequent in cats.

Protocol • When we perform the TRH stimulation test in our hospital, 0.2 mg of TRH/dog is administered IV, and blood for serum total T_4 determination is obtained before and 4 hours after TRH administration. When this test is performed in cats, blood is collected for serum T_4 determination before and 4 hours after IV administration of 0.1 mg of TRH/kg of body weight. Adverse reactions (i.e., increased salivation, urination, defecation, vomiting, miosis, tachycardia, tachypnea) are common in cats and may also occur in dogs when TRH dose exceeds 0.1 mg/kg. Adverse reactions usually begin immediately after TRH administration and may continue for 4 hours.

Interpretation • Interpretation of the TRH stimulation test is more subjective than for the TSH stimulation test, in part because increase in serum total T_4 concentration is considerably less dramatic with TRH than TSH. Using the TRH protocol described earlier, euthyroid dogs should have a post-TRH serum T_4 concentration greater than 2 µg/dl. Alternatively, post-TRH serum T_4 should increase at least 0.5 µg/dl above baseline serum T_4 concentration in a euthyroid dog. In contrast, dogs with primary hypothyroidism should have a post-TRH serum T_4 concentration below normal baseline serum T_4 range (i.e., < 1.5 µg/dl), and there should be less than a 0.5 µg/dl increase in serum T_4 after TRH administration. Post-TRH serum T_4 concentrations of 1.5 to 2.0 µg/dl are nondiagnostic and occur in early hypothyroidism or may represent suppression of thyroid gland function because of concurrent illness or drug therapy in an otherwise euthyroid dog.

Healthy cats and those with nonthyroidal disease consistently demonstrate a twofold increase in serum T_4 concentration 4 hours after IV administration of TRH (Peterson, Broussard, and Gamble, 1994). A single administration of TRH should not increase pituitary TSH secretion in hyperthyroid cats. Consequently, cats with mild hyperthyroidism have little or no increase in serum T_4 concentrations. An increase in post-TRH serum T_4 concentration of less than 50% above baseline values is also consistent with hyperthyroidism. Post-TRH T_4 values greater than 60% above baseline support a normal pituitary-thyroid axis. Increases of 50% to 60% are nondiagnostic.

PITUITARY ADRENOCORTICOTROPIN HORMONE

Occasionally Indicated • In dogs, pituitary ACTH concentration is determined to differentiate pituitary-dependent from adrenal-dependent spontaneous hyperadrenocorticism and to differentiate primary versus secondary hypoadrenocorticism. Similar indications exist for cats, although baseline ACTH concentration can be less than 10 pg/ml in normal cats and cats with hyperadrenocorticism caused by adrenocortical tumor (AT) (Smith and Feldman, 1987). Measurement of baseline endogenous ACTH concentration is not used to diagnose hyperadrenocorticism because of episodic secretion and overlapping values between normal and pituitary-dependent hyperadrenocorticism (PDH).

Advantage • It is a reliable test to differentiate PDH from AT.

Disadvantages • ACTH is a fragile hormone, requiring meticulous care in specimen handling (see later). In addition, the test is expensive, has limited availability, and plasma samples must remain frozen until assayed.

Protocol • A blood sample should be drawn between 8 and 9 AM, preferably after overnight hospitalization. Blood should be collected in a cold heparinized plastic syringe, immediately transferred to cold plastic tubes, and placed on ice until centrifuged. Alternatively, blood can be placed in cooled, silicone-coated EDTA tubes and placed on ice. Blood should be centrifuged immediately and plasma frozen in plastic tubes. This entire procedure should take less than 10 minutes. Samples must be shipped frozen and must stay frozen until assayed for ACTH. Validated ACTH assays are currently limited by availability and cost.

Analysis • ACTH is measured in plasma by RIA. The assay must be validated for the dog and cat.

Normal Values • Dogs, 10 to 110 pg/ml; cats, undetectable to 110 pg/ml. To convert from pg/ml to pmol/L, multiply by 0.220.

Interpretation in dogs with hyperadrenocorticism:
Less than 10 pg/ml: adrenal-dependent hyperadrenocorticism
10 to 45 pg/ml: nondiagnostic
Greater than 45 pg/ml: pituitary-dependent hyperadrenocorticism

Interpretation in dogs with hypoadrenocorticism:
Less than 10 pg/ml: secondary hypoadrenocorticism
10 to 45 pg/ml: nondiagnostic
Greater than 45 pg/ml: primary hypoadrenocorticism

Danger Values • None.

Artifacts • Falsely decreased: storing plasma above freezing, sample thawing during transport to laboratory, using glass containers during collection or storage.

Drug Therapy That May Alter ACTH Values • Decreased: administration of glucocorticoids (e.g., dexamethasone). Increased: insulin.

Causes of Altered Plasma ACTH Concentration • Alterations in baseline endogenous ACTH concentration result from primary pituitary gland disorders or disorders affecting blood cortisol concentration with its negative inhibitory effects on ACTH secretion (Table 8-12). Pituitary disorders include PDH, which is associated with increased ACTH secretion (Figure 8-10), and secondary hypoadrenocorticism, which results from loss of function of the pituitary corticotroph cells and decreased ACTH secretion (Figure 8-11). Functional ATs of the zona fasciculata have increased cortisol secretion, which inhibits pituitary ACTH secretion; plasma ACTH typically decreases to undetectable concentrations (see Figure 8-10). A similar phenomenon occurs with excessive exogenous glucocorticoid administration (i.e., iatrogenic hyperadrenocorticism). Destruction of the zona fasciculata (e.g., primary hypoadrenocorticism) decreases

TABLE 8-12. Causes of Altered Endogenous Plasma Adrenocorticotropic Hormone (ACTH) Concentration in Dogs

Normal to increased
Pituitary-dependent hyperadrenocorticism
Primary hypoadrenocorticism
Normal to decreased
Adrenal-dependent hyperadrenocorticism
Iatrogenic hyperadrenocorticism
Spontaneous secondary hypoadrenocorticism*
Improper sample collection/storage

*Rare.

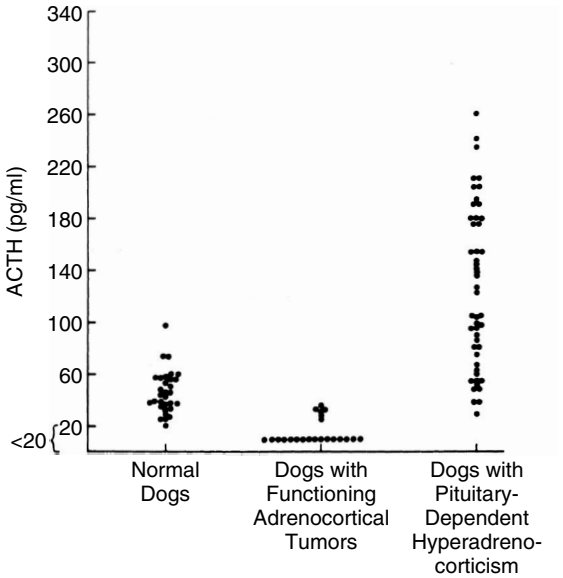

FIGURE 8-10. Endogenous plasma adrenocorticotropic hormone *(ACTH)* concentrations from clinically normal dogs, dogs with adrenal-dependent hyperadrenocorticism (adrenocortical carcinomas or adenomas), and dogs with pituitary-dependent hyperadrenocorticism. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction,* Philadelphia, 1987, WB Saunders.)

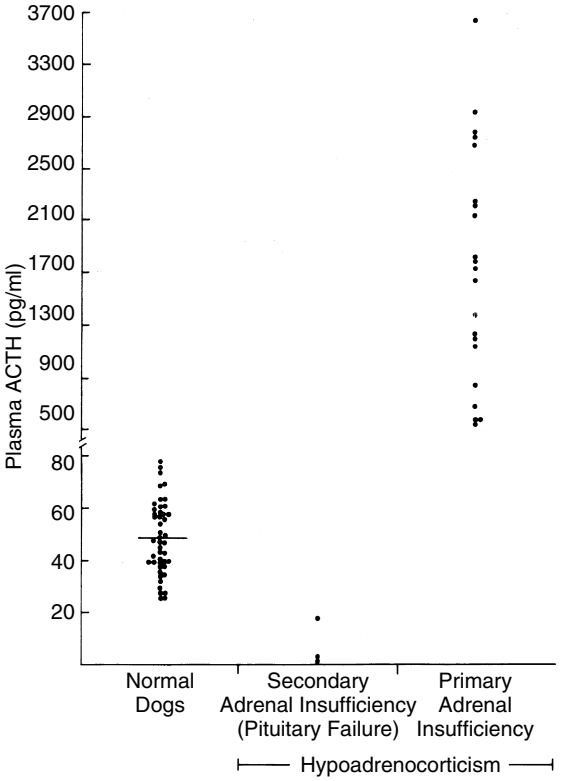

FIGURE 8-11. Endogenous plasma adrenocorticotropic hormone *(ACTH)* concentrations in normal dogs, dogs with secondary adrenal failure, and dogs with primary adrenal failure. (From Feldman EC, Peterson ME: Hypoadrenocorticism, *Vet Clin North Am* 14:761, 1984.)

blood cortisol concentration, thus increasing plasma ACTH concentrations (see Figure 8-11). Preliminary evaluation of assays for endogenous ACTH in cats shows similar results (Peterson, Greco, and Orth, 1989), although values in normal cats can be less than 10 pg/ml. Similar results can occur with feline ATs, making usefulness of this assay questionable in cats.

PLASMA CORTISOL

Commonly Indicated • Plasma cortisol is measured to assess the pituitary adrenocortical axis in patients with suspected hyperadrenocorticism or hypoadrenocorticism. Findings suggestive of hyperadrenocorticism include polydipsia, polyuria, polyphagia, endocrine alopecia, panting, weakness, calcinosis cutis, epidermal and dermal atrophy, conformational changes (e.g., "potbellied appearance"), insulin-resistant diabetes mellitus, hepatomegaly, stress leukogram, increased SAP, hypercholesterolemia, persistent hyposthenuria, and recurring urinary tract infection, especially in middle-aged to older patients. Findings suggestive of hypoadrenocorticism include lethargy, depression, anorexia, vomiting, weakness, weight loss, bradycardia, hypovolemia, hyponatremia, and hyperkalemia, especially in young to middle-aged dogs and cats.

Advantages • The test is readily available, and the hormone is stable.

Disadvantages • Baseline plasma cortisol concentration has no diagnostic significance; interpretation is only valid after manipulation of the pituitary-adrenocortical axis with ACTH or dexamethasone.

Analysis • Plasma cortisol is measured in heparinized or EDTA plasma by RIA or in-house ELISAs. RIAs measure free and protein-bound cortisol. RIAs should be validated for the dog and cat. Plasma should be harvested and frozen soon after collection to minimize binding of cortisol to RBCs. If mailed, plasma samples should be shipped with cool packs. Repeated freezing and thawing or hemolysis of the

sample does not alter cortisol concentrations. Fasting for up to 36 hours does not affect cortisol values, and a clinically detectable diurnal variation does not exist in the dog or cat.

Normal Values • Dogs, 1.0 to 6.0 µg/dl; cats, 1.0 to 5.0 µg/dl. To convert from µg/dl to nmol/L, multiply by 27.59.

Danger Values • None.

Artifacts Affecting Plasma Cortisol Concentration • Environment (e.g., "stress," excitement) and chronic disease may increase baseline plasma cortisol concentrations. Exogenous glucocorticoid preparations containing hydrocortisone, cortisone, prednisone, prednisolone, and possibly methylprednisolone cross-react with many cortisol assays, causing spurious increases in measured cortisol values. Dexamethasone does not cross-react with cortisol assays. Plasma cortisol concentrations decrease by 21% after 2 days and 57% after 8 days at 22°C but do not decrease when stored at 4°C for 8 days.

Drug Therapy That May Alter Plasma Cortisol • Estrogen administration may increase plasma cortisol. Chronic androgen or glucocorticoid administration and progestagens such as megestrol acetate can decrease plasma cortisol concentration.

Causes of Hypercortisolemia • The most clinically relevant cause of hypercortisolemia is hyperadrenocorticism (Table 8-13).

TABLE 8-13. Causes of Altered Resting Plasma Cortisol Concentrations in Dogs and Cats

Increased
Stress
Severe or chronic illness
Drugs
Cortisone, hydrocortisone, prednisone and prednisolone (due to cross-reaction with cortisol assay)
Anticonvulsants
Hyperadrenocorticism
Pituitary-dependent
Adrenal-dependent
Decreased
Improper storage
Iatrogenic hyperadrenocorticism
Hypoadrenocorticism
Primary
Secondary (i.e., pituitary insufficiency)
Drugs
Progestagens
Megestrol acetate

Additional causes include environmental factors that create "stress" or excitement, chronic illness (e.g., diabetes mellitus, renal insufficiency, congestive heart failure), and medications that contain glucocorticoid preparations that cross-react with the cortisol assay. For the latter to cause increased cortisol values, medication must have been given within 12 to 24 hours of blood sampling and still be present in blood. Once glucocorticoid in the preparation has been metabolized, plasma cortisol concentrations decrease because of the negative inhibitory effects of exogenous glucocorticoid on pituitary ACTH secretion. This phenomenon (i.e., iatrogenic hyperadrenocorticism) creates signs of hyperadrenocorticism, but the pituitary-adrenocortical axis is suppressed and resembles hypoadrenocorticism (Figure 8-12; see Causes of Hypocortisolemia).

Hyperadrenocorticism occurs in dogs and rarely in cats. A tentative diagnosis of hyperadrenocorticism can be established based on history, physical examination, and routine clinical pathologic assessments (e.g., CBC, serum biochemical panel, urinalysis; see description under the Commonly Indicated heading, under Plasma Cortisol). Common abnormalities identified on clinical pathologic assessments include a stress leukogram,

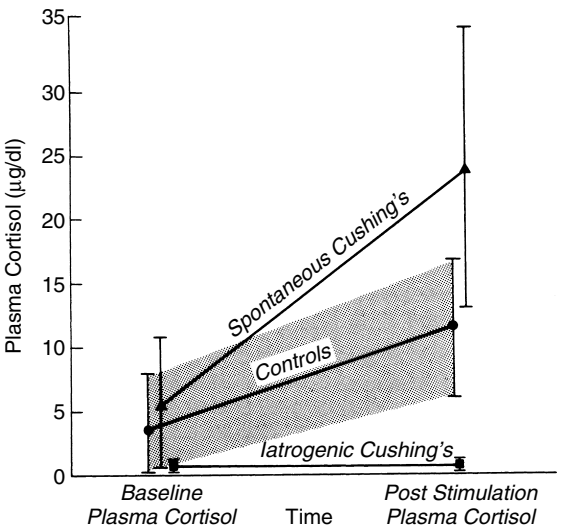

FIGURE 8-12. Mean plasma cortisol concentrations (± 2 SD) determined before and 1 hour after the administration of synthetic adrenocorticotropic hormone (ACTH) in control dogs, dogs with spontaneous hyperadrenocorticism, and dogs with iatrogenic hyperadrenocorticism. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, ed 2, Philadelphia, 1996, WB Saunders.)

increased SAP and ALT activities, hypercholesterolemia, and isosthenuria to hyposthenuria, proteinuria, and bacteriuria. In cats with hyperadrenocorticism, hyperglycemia and hypercholesterolemia are the most consistent clinical pathologic findings (Nelson, Feldman, and Smith, 1988). Additional abnormalities in both species include increased serum amylase, lipase, and insulin concentrations and decreased baseline serum T_4, fT_4, T_3, and possibly TSH concentrations. Many dogs with hyperadrenocorticism are misdiagnosed with primary hepatic disease because of hepatomegaly, increased hepatic enzymes (especially SAP), increased serum bile acid concentrations, and vacuolar hepatopathy.

Diagnosis of hyperadrenocorticism and differentiation between PDH and AT requires evaluation of plasma cortisol concentrations after manipulation of the pituitary-adrenocortical axis with ACTH or dexamethasone (Figure 8-13). Baseline plasma cortisol concentration has no diagnostic value. Tests to confirm hyperadrenocorticism include the ACTH-stimulation test, low-dose dexamethasone suppression (LDDS) test, and combination ACTH-dexamethasone suppression test. Tests to differentiate between PDH and AT include endogenous ACTH concentration, LDDS test, high-dose dexamethasone suppression (HDDS) test, and abdominal ultrasonography. Diagnosis of iatrogenic hyperadrenocorticism is based on a history of glucocorticoid administration and ACTH-stimulation test results. The reader is referred to appropriate headings that follow for specific information on these tests.

The most commonly used tests to diagnose hyperadrenocorticism in dogs and to differentiate PDH from AT are the ACTH stimulation test, LDDS test, and abdominal ultrasonography. The urine cortisol:creatinine ratio can be assessed as part of the initial screen for hyperadrenocorticism or, more commonly, is assessed to further rule out hyperadrenocorticism if results of previously listed tests are normal or inconclusive and the dog has ambiguous clinical signs. Because adrenalectomy is currently the only reliable long-term treatment option for hyperadrenocorticism in cats, differentiating PDH from AT is not as important as in dogs. We routinely use the dexamethasone suppression test, urine cortisol: creatinine ratio, and abdominal ultrasonography to establish the diagnosis of hyperadrenocorticism in cats. In the authors' experience, the sensitivity of the ACTH-stimulation test has been less than 50% in cats with hyperadrenocorticism.

Causes of Hypocortisolemia • The most clinically relevant cause of hypocortisolemia is primary hypoadrenocorticism (see Table 8-13), caused by destruction of the adrenal zona glomerulosa (mineralocorticoid-producing zone) and zona fasciculata (glucocorticoid-producing zone). Additional causes include iatrogenic hyperadrenocorticism (see Causes of Hypercortisolemia), secondary hypoadrenocorticism, progesterone-secreting ATs, megestrol acetate therapy, and improper sample handling. Secondary hypoadrenocorticism results from a selective deficiency of glucocorticoid secretion, which is usually caused by inadequate ACTH secretion as the result of pituitary disease.

Primary hypoadrenocorticism typically occurs in young adult dogs and rarely in cats. History, physical examination, and routine clinical pathologic assessments often suggest hypoadrenocorticism. Clinical pathologic abnormalities may include mild, nonregenerative anemia, lack of a stress leukogram in a sick dog or cat, hyperkalemia, hyponatremia, hypochloremia, azotemia, hyperphosphatemia, mild hypercalcemia (i.e., 12 to 14 mg/dl), mild hypoglycemia (i.e., 45 to 60 mg/dl), and metabolic acidosis. Urine specific gravity can range from hypersthenuric to isosthenuric (because of renal sodium wasting and loss of the renal medullary concentration gradient) and so can mimic primary renal disease. Cardiac conduction disturbances may develop with severe hyperkalemia (i.e., >7 mEq/L). If present, these disturbances are readily identified on a lead II rhythm strip of an ECG and may include dampened P wave, prolonged PR interval and QRS complex, spiked T wave, and ventricular arrhythmias.

Hallmark abnormalities identified on clinical pathologic assessments are hyperkalemia, hyponatremia, and hypochloremia. The sodium:potassium ratio reflects changes in these electrolytes. Values are often well below 27:1 in primary hypoadrenocorticism (normal ratios 27:1-40:1). Normal serum electrolyte concentrations, however, do not rule out adrenal insufficiency. Serum electrolyte concentrations may be normal in early disease, in selective hypocortisolism, in hypoadrenal patients that have recently received fluid therapy, and in secondary hypoadrenocorticism. Furthermore, other disorders (especially renal, gastrointestinal, and hepatic) can cause

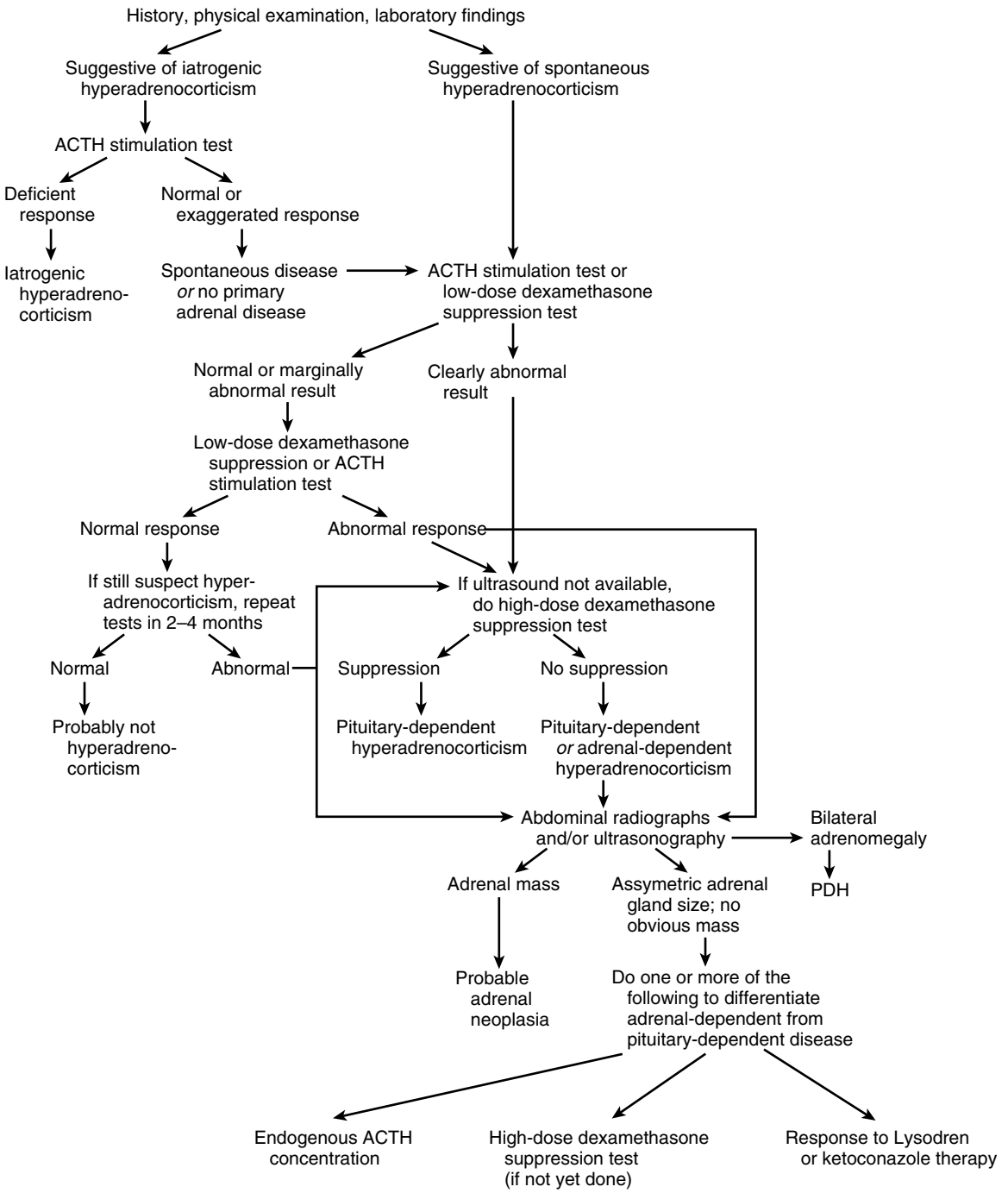

FIGURE 8-13. Evaluation of patients with suspected hyperadrenocorticism based on history (polyuria-polydipsia, polyphagia, anestrus, panting), physical examination (truncal alopecia, pot-belly, calcinosis cutis, hepatomegaly), or laboratory findings (increased serum alkaline phosphatase [SAP], lymphopenia, eosinopenia, hypercholesterolemia, urinary tract infection). *ACTH*, Adrenocorticotropic hormone.

serum electrolyte changes mimicking adrenal insufficiency. The reader should refer to Chapter 6 for other causes of hyponatremia, hypochloremia, and hyperkalemia.

Confirmation of hypoadrenocorticism requires an ACTH-stimulation test (Figure 8-14). Animals with adrenal insufficiency have low or low-normal baseline plasma cortisol

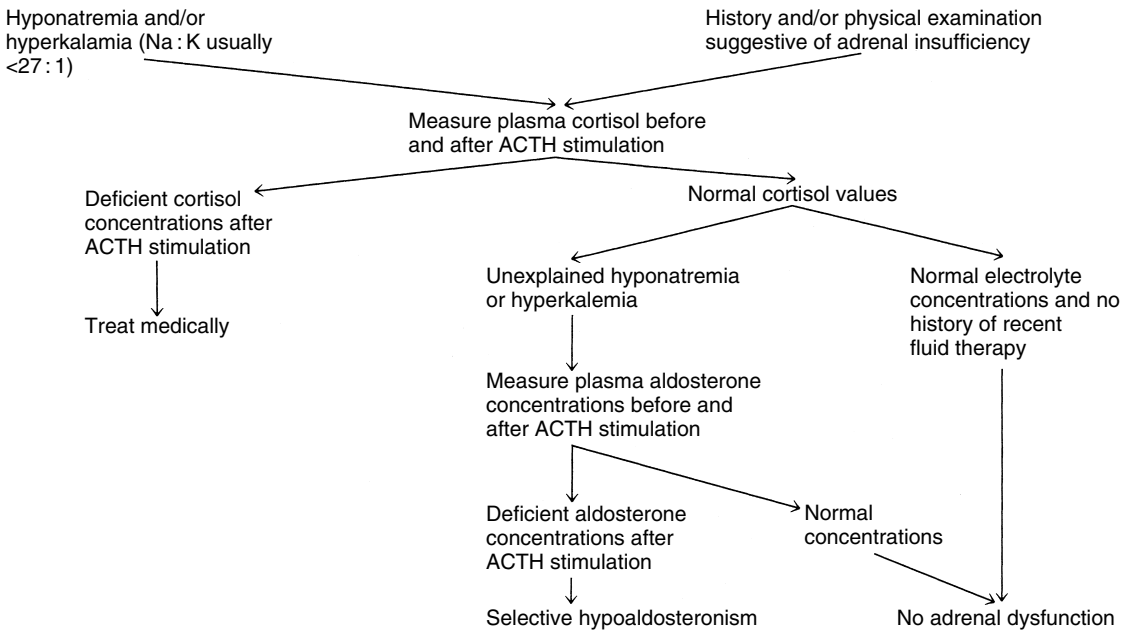

FIGURE 8-14. Diagnostic evaluation of dogs and cats with suspected hypoadrenocorticism and hypocortisolism. *ACTH*, Adrenocorticotropic hormone.

concentration and minimal to no increase in plasma cortisol after the administration of ACTH (Figure 8-15). The ACTH-stimulation test does not distinguish primary from secondary adrenal insufficiency. Concurrent electrolyte abnormalities imply primary hypoadrenocorticism, but normal electrolyte concentrations do not differentiate early primary from secondary insufficiency. Differentiation requires measurement of baseline endogenous ACTH concentration or plasma aldosterone concentrations during the ACTH stimulation test (see Figure 8-14 and Plasma Aldosterone).

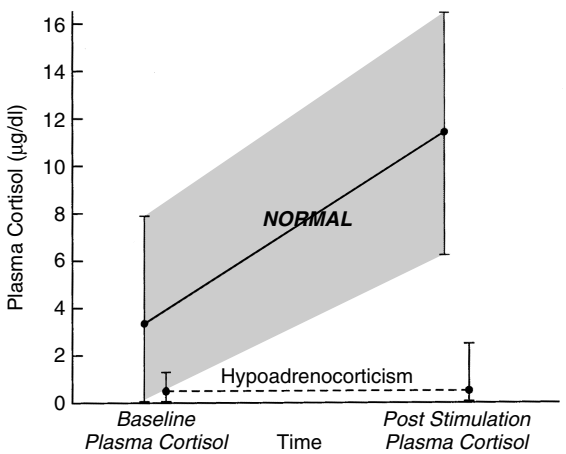

FIGURE 8-15. Plasma cortisol concentrations before and after exogenous adrenocorticotropic hormone (ACTH) stimulation in normal dogs and in dogs with hypoadrenocorticism. The ranges are means ±2 SD. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, ed 2, Philadelphia, 1996, WB Saunders.)

ADRENOCORTICOTROPIN HORMONE STIMULATION TEST

Commonly Indicated • An ACTH-stimulation test is done to confirm hypoadrenocorticism and iatrogenic hyperadrenocorticism, to screen for spontaneous hyperadrenocorticism, and to monitor mitotane (*o,p'*-DDD) therapy in dogs with PDH. The ACTH-stimulation test does not differentiate between PDH and AT.

Advantages • The test is readily available, relatively inexpensive, and easy to interpret.

Disadvantages • Results may be affected (i.e., exaggerated) by chronic illness and "stress"; the test does not differentiate between primary and secondary hypoadrenocorticism.

Protocol • The protocol for the ACTH-stimulation test differs between the dog and

cat and with the type of ACTH used. When ACTH gel is used, blood for cortisol assay is obtained before and 2 hours after (dog) and before and 1 and 2 hours after (cat) intramuscular (IM) administration of 2.2 IU ACTH gel/kg body weight. When synthetic ACTH is used, blood for cortisol assay is obtained before and 1 hour after IM administration of 0.25 mg synthetic ACTH (irrespective of weight [dogs]) and before, 30 and 60 minutes after IM administration of 0.125 mg synthetic ACTH (irrespective of weight [cats]). The ACTH-stimulation test can be performed any time during the day.

Artifacts and Drug Therapy That May Alter Results • Anything altering plasma cortisol concentration can affect ACTH-stimulation test results (see Artifacts That May Alter Plasma Cortisol Concentration). Anticonvulsant medications may spuriously increase ACTH-stimulation test results.

Interpretation in dogs:

Post-ACTH cortisol, less than 5 μg/dl: hypoadrenocorticism or iatrogenic hyperadrenocorticism
Post-ACTH cortisol, 6 to 18 μg/dl: normal
Post-ACTH cortisol, 18 to 24 mg/dl: suggestive of spontaneous hyperadrenocorticism
Post-ACTH cortisol, greater than 24 μg/dl: strongly suggestive of spontaneous hyperadrenocorticism

Interpretation in cats:

Post-ACTH cortisol, less than 5 μg/dl: hypoadrenocorticism or iatrogenic hyperadrenocorticism
Post-ACTH cortisol, 6 to 12 μg/dl: normal
Post-ACTH cortisol, 13 to 16 μg/dl: suggestive of spontaneous hyperadrenocorticism
Post-ACTH cortisol, greater than 16 μg/dl: strongly suggestive of spontaneous hyperadrenocorticism

To convert mg/dl to nmol/L, multiply by 27.59.

LOW-DOSE DEXAMETHASONE SUPPRESSION TEST

Commonly Indicated • A LDDS test is done to screen for spontaneous hyperadrenocorticism and to differentiate between PDH and AT. This test does not identify iatrogenic hyperadrenocorticism, nor is it used to assess response to mitotane or ketoconazole therapy.

Advantages • The test is readily available, relatively inexpensive, and easy to interpret. It often confirms hyperadrenocorticism and identifies PDH at the same time.

Disadvantages • Results can be affected by acute "stress" and concurrent illness. In addition, other procedures must be avoided until test completion (about 8 hours).

Protocol • Ideally, this test should be started between 8 and 9 AM after the patient has been hospitalized overnight. The patient should rest quietly in the cage except when walked outside or blood is taken. In the dog, a plasma sample for cortisol analysis is obtained immediately before and 4 and 8 hours after IV administration of 0.01 mg dexamethasone/kg body weight. The same dose of dexamethasone is used in the cat, although blood samples are obtained immediately before and 4, 6, and 8 hours after IV dexamethasone administration. Dexamethasone sodium phosphate or dexamethasone in polyethylene glycol can be used.

Analysis and Artifacts • See Plasma Cortisol.

Artifacts and Drug Therapy That May Alter Results • Anything altering plasma cortisol concentration can affect LDDS test results (see Artifacts That May Alter Plasma Cortisol Concentration). Results may be affected by concurrent anticonvulsant drugs, stress (e.g., bathing, concurrent diagnostics), exogenous glucocorticoids, and nonadrenal disease; the more severe the nonadrenal disease, the more likely the chance of obtaining false-positive test results.

Interpretation • Evaluation of the 8-hour post-dexamethasone plasma cortisol concentration is used to confirm hyperadrenocorticism (Figure 8-16). Normal dogs have plasma cortisol values less than 1.0 μg/dl, whereas dogs with PDH and AT have plasma cortisol concentrations greater than or equal to 1.4 μg/dl at 8 hours. Cortisol concentrations of 1.0 to 1.4 μg/dl are nondiagnostic, and the clinician must consider other information to diagnose hyperadrenocorticism.

If the 8-hour post-dexamethasone cortisol value supports hyperadrenocorticism, the 4-hour post-dexamethasone plasma cortisol value may be useful in distinguishing between PDH and AT (Mack and Feldman, 1990).

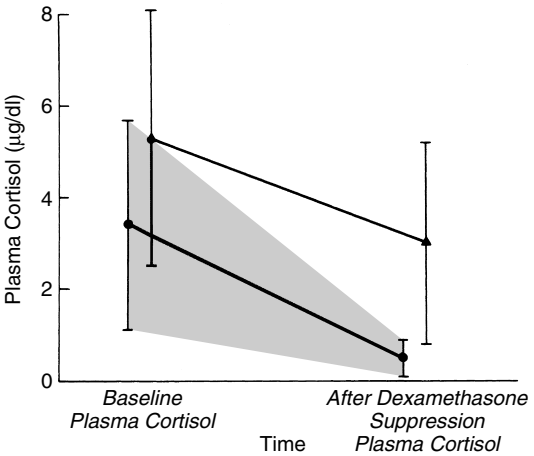

FIGURE 8-16. Mean plasma cortisol concentrations (±2 SD) determined before and 8 hours after administration of a low dexamethasone dose (0.01 mg/kg) intravenously (IV) in control dogs (•) and in dogs with hyperadrenocorticism (▲). The reader should note the slight overlap of values after dexamethasone. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, Philadelphia, 1987, WB Saunders.)

Low doses of dexamethasone suppress pituitary ACTH secretion and plasma cortisol concentrations in approximately 60% of dogs with PDH during the initial 2 to 6 hours of the test. Suppression does not occur in dogs with AT, nor does it occur in approximately 40% of dogs with PDH. Suppression is defined as (1) a 4-hour post-dexamethasone plasma cortisol concentration less than 1.4 µg/dl, (2) a 4-hour post-dexamethasone plasma cortisol concentration less than 50% of baseline concentration, and (3) an 8-hour post-dexamethasone plasma cortisol concentration less than 50% of baseline concentration (Feldman, Nelson, and Feldman, 1996). Any hyperadrenal dog with plasma cortisol concentrations meeting one or more of these three criteria usually has PDH. Failure to meet any of these criteria is consistent with lack of suppression. Lack of suppression is nonspecific, consistent with hyperadrenocorticism but not distinguishing between pituitary and adrenal disease.

This test is difficult to interpret in cats. An occasional normal cat will "escape" the suppressive effects of 0.01 mg dexamethasone/kg given IV and fall outside the normal 8-hour post-dexamethasone reference range (i.e., 8-hour post-dexamethasone plasma cortisol >1.4 µg/dl) (Smith and Feldman, 1987). A plasma cortisol concentration greater than 1.4 µg/dl at 4, 6, and 8 hours strongly suggests hyperadrenocorticism. The test is inconclusive if either the 4- or 6-hour cortisol value is less than 1.4 µg/dl and the 8-hour value is greater than 1.4 µg/dl. These results suggest hyperadrenocorticism but may also occur in normal cats that have escaped the suppressive effects of dexamethasone. The test should be repeated using 0.1 mg dexamethasone/kg (see High-Dose Dexamethasone Suppression Test). Because of potential for escape in normal cats, the LDDS test should never be the only test used to confirm diagnosis of feline hyperadrenocorticism.

COMBINED DEXAMETHASONE SUPPRESSION–ADRENOCORTICOTRIPIN HORMONE STIMULATION TEST

Rarely Indicated • This test was used to screen for hyperadrenocorticism. It does not reliably differentiate PDH from AT and should not be used for this purpose.

Advantages • The test is readily available, relatively quick, and easy to interpret.

Disadvantages • It is more expensive and less reliable than ACTH stimulation test or LDDS test; chronic illness and "stress" may affect results. This test is *not recommended*.

HIGH-DOSE DEXAMETHASONE SUPPRESSION TEST

Infrequently Indicated • A HDDS test is done to distinguish PDH from AT in dogs with confirmed spontaneous hyperadrenocorticism and to confirm hyperadrenocorticism in cats. The need for this test has declined because of the increasing availability of abdominal ultrasonography to evaluate adrenal glands.

Protocol • Ideally, this test should be started between 8 and 9 AM after the patient has been hospitalized overnight. The patient should rest quietly in the cage except when walked outside or when blood is obtained. In dogs, a plasma sample for cortisol analysis is obtained immediately before and 8 hours after IV administration of 0.1 mg dexamethasone/kg body weight. Obtaining a 4-hour post-dexamethasone blood sample is optional; in the authors' experience, the 4-hour HDDS sampling was

informative in only 2% of dogs tested with both LDDS and HDDS (Feldman, Nelson, and Feldman, 1996). The same dose of dexamethasone is used in cats, although blood samples are obtained immediately before and 4, 6, and 8 hours after IV dexamethasone administration. Dexamethasone sodium phosphate or dexamethasone in polyethylene glycol can be used.

Interpretation • A higher dose of dexamethasone is used in an attempt to suppress pituitary ACTH secretion in dogs with PDH. Suppression is defined as a 4-hour or 8-hour post-dexamethasone plasma cortisol concentration less than 1.4 μg/dl, and a 4-hour or 8-hour post-dexamethasone plasma cortisol concentration less than 50% of baseline concentration. Any dog with hyperadrenocorticism and plasma cortisol concentrations meeting one or more of these four criteria most likely has PDH. Approximately 75% of dogs with PDH meet at least one of the four criteria for "suppression" on the HDDS test. Failure to meet any of these criteria is consistent with lack of suppression. Among dogs with hyperadrenocorticism in which suppression is not demonstrated are approximately 25% of dogs with PDH and almost 100% of dogs with ATs (Figure 8-17). Higher doses of dexamethasone (e.g., 1.0 mg/kg) could be administered in an attempt to suppress pituitary ACTH secretion in dogs with dexamethasone-resistant PDH. The percentage of dogs with PDH suppression with higher doses of dexamethasone is similar to that with the 0.1 mg/kg protocol, however.

The HDDS test is also used to diagnose feline hyperadrenocorticism. In normal cats, the plasma cortisol concentrations should be less than 1.4 μg/dl at 4, 6, and 8 hours. Similar results occur in some cats with PDH. Hyperadrenocorticism should be suspected if the 8-hour plasma cortisol concentration is greater than 1.4 μg/dl. The likelihood of this disease is increased further if the 4- and 6-hour plasma cortisol concentration is also greater than 1.4 μg/dl. Any post-dexamethasone plasma cortisol concentration less than 50% precortisol value supports PDH once hyperadrenocorticism is confirmed.

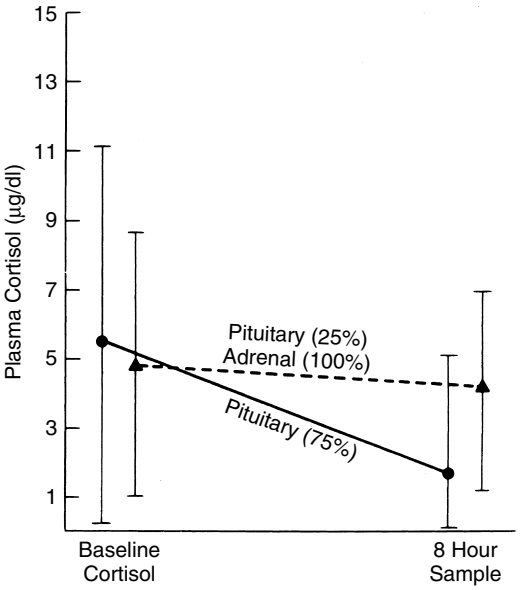

FIGURE 8-17. Patterns of plasma cortisol responses during high-dose (0.1 mg/kg) dexamethasone suppression testing in dogs with pituitary-dependent or adrenal-dependent hyperadrenocorticism. The reader should note that suppression is diagnostic of pituitary dependency. Lack of suppression occurs in all adrenal tumor cases and in 25% of pituitary-dependent cases. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, Philadelphia, 1987, WB Saunders.)

URINE CORTISOL:CREATININE RATIO

Indications • A urine cortisol:creatinine ratio is done as a screening test for hyperadrenocorticism. Dogs with hyperadrenocorticism have increased 24-hour urinary free cortisol excretion compared with healthy dogs (Stolp et al, 1983). Urine cortisol is measured by routine cortisol RIA technique on unextracted urine. The urine cortisol:creatinine ratio on a random urine sample is significantly increased in dogs with spontaneous hyperadrenocorticism compared with healthy dogs (Feldman and Mack, 1992). Unfortunately, the ratio is increased in dogs with nonadrenal illness and in those with clinical signs consistent with hyperadrenocorticism but with a normal pituitary-adrenocortical axis.

Protocol • A free-catch urine sample is submitted to the laboratory for determination of cortisol and creatinine concentrations. It is preferable to have the owner collect the urine at home with the dog in a "nonstressed" state because stress associated with driving the dog to the veterinary hospital and undergoing a physical examination before collecting urine can affect test results. The urine cortisol:creatinine ratio is determined by dividing urine cortisol concentration (in μmol/L) by urine creatinine concentration (in μmol/L).

Interpretation • In the authors' laboratory, the urine cortisol:creatinine ratio is less than 1.35×10^{-5} in normal dogs. A normal urine cortisol:creatinine ratio is inconsistent with hyperadrenocorticism. A urine cortisol:creatinine ratio greater than 1.35×10^{-5} is consistent with hyperadrenocorticism and supports the performance of additional diagnostics (e.g., LDDS test) to confirm the diagnosis, assuming history, physical examination, and results of routine blood work also support a diagnosis of hyperadrenocorticism. In one study, the specificity of the urine cortisol:creatinine ratio was only 20% when sick dogs without hyperadrenocorticism were compared with dogs with hyperadrenocorticism (Smiley and Peterson, 1993). Thus, although a normal urine cortisol:creatinine ratio essentially eliminates hyperadrenocorticism and is used as a screening test for normalcy, an increased urine cortisol:creatinine ratio is not diagnostic for spontaneous hyperadrenocorticism. An increased urine cortisol:creatinine ratio does not, by itself, confirm a diagnosis of hyperadrenocorticism. Dogs with other illnesses or stress commonly have increased urine cortisol:creatinine ratios (results that overlap with those obtained from dogs with hyperadrenocorticism).

The normal reference range for urine cortisol:creatinine ratio in cats is 0.2 to 3.6×10^{-5} (median, 1.3×10^{-5}). Interpretation of the urine cortisol:creatinine ratio is similar to that in dogs (i.e., a normal ratio rules out hyperadrenocorticism, whereas an increased ratio is consistent with, but not definitive for, hyperadrenocorticism).

PLASMA ALDOSTERONE

Rarely Indicated • Plasma aldosterone is measured to (1) identify selective aldosterone deficiency in patients with hyponatremia, hyperkalemia, and normal plasma cortisol responsiveness to ACTH; (2) distinguish primary adrenal disease from secondary adrenal atrophy because of a pituitary or hypothalamic deficiency in dogs with normal serum electrolytes and hypoadrenal ACTH-stimulation test results; (3) identify hyperaldosteronism; and (4) assess hormonal function of an adrenal mass. Clinical findings with hyperaldosteronism include lethargy, weakness, hypokalemia, hypernatremia, hypertension, and adrenomegaly.

Analysis • Aldosterone is measured in EDTA or heparinized plasma, or in serum by RIA. In the authors' laboratory, serum and heparinized plasma yield comparable results. Baseline aldosterone concentration has little to no diagnostic value. Serum aldosterone is always interpreted after stimulation with ACTH (see ACTH-Stimulation Test). Timing of blood sampling is the same as for plasma cortisol. For meaningful results, the aldosterone assay must be validated for use in each species to be tested and normal values established. See Appendix I for availability of testing. Handling and shipping are as described for cortisol.

Normal Serum Values in Dogs • Baseline in dogs: mean, 49 pg/ml; range, 2 to 96 pg/ml. One hour postsynthetic ACTH administration: mean, 306 pg/ml; range, 146 to 519 pg/ml. To convert pg/ml to ng/dl, divide result by 10. To convert pg/ml to pmol/L, multiply by 2.775.

Danger Values • None.

Artifacts • Storage at 22° C for greater than or equal to 3 days or at 37° C for 1 day decreases measured plasma aldosterone concentration. High sodium intake may suppress, whereas low sodium intake may increase serum aldosterone concentrations.

Interpretation • Hypoaldosteronism is documented by the finding of a low baseline value and minimal to no increase in post-ACTH plasma aldosterone concentration (Golden and Lothrop, 1988). In theory, measurement of blood aldosterone should be valuable when attempting to distinguish dogs with primary adrenal disease from those with secondary adrenal atrophy because of a pituitary or hypothalamic deficiency. Unfortunately, no clear demarcation in blood aldosterone concentrations exists between these groups. After ACTH administration, mean serum aldosterone concentration was 13 pg/ml (range, 0.1 to 91 pg/ml) in 15 dogs with naturally occurring primary hypoadrenocorticism and 28 and 41 pg/ml in two dogs with secondary hypoadrenocorticism. Markedly increased baseline and post-ACTH plasma aldosterone concentrations suggest primary hyperaldosteronism in dogs with no other explanation for hypokalemia, hyperkaluria, hypernatremia, decreased natruresis, and systemic hypertension. Identification of unilateral adrenomegaly

with abdominal ultrasonography further supports the diagnosis.

INSULIN-LIKE GROWTH FACTOR-I

Infrequently Indicated • Measurement of insulin-like growth factor-I (IGF-I) concentration is done as a screening test for acromegaly in diabetic cats with insulin resistance. Most of the growth-promoting effects of growth hormone (GH) are mediated by IGF-I. The liver is a major site of IGF-I synthesis, and much of the circulating IGF-I is believed to be derived from the liver. IGF-I has an approximately 50% homology of structure with proinsulin and insulin, and the IGF-I cell membrane receptor resembles the insulin receptor in its structure. Secretion of IGF-I is under direct control of GH. As such, serum IGF-I concentrations are increased in acromegaly.

Analysis • IGF-I is measured in serum by RIA.

Normal Values • Cats, 5 to 70 nmol/L.

Artifacts • Unknown.

Drug Therapy That May Alter Serum IGF-I • Unknown.

Role as a Diagnostic Test for Feline Acromegaly • Increased IGF-I concentrations have been found in acromegalic cats (Middleton et al, 1985; Abrams-Ogg et al, 1993), suggesting that IGF-I may be a useful diagnostic tool. However, the role of IGF-I as a screen for GH excess was questioned in one study in which serum IGF-I concentrations were fourfold higher in eight randomly selected diabetic cats, compared with eight healthy cats (Lewitt et al, 2000). None of the diabetic cats were suspected to have acromegaly. In the authors' experience, serum IGF-I concentration has proven to be a useful screening test for GH excess when evaluated in diabetic cats with insulin resistance. Most cats with acromegaly will have serum IGF-I concentrations greater than 100 nmol/l. Serum IGF-I values between 70 and 100 nmol/l are nondiagnostic. Some nonacromegalic diabetic cats with insulin resistance have serum IGF-I values that fall in this range, and some acromegalic diabetic cats have serum IGF-I values that fall in the upper-normal range in the early stages of the disease. Repeat measurements performed 3 to 6 months later will usually demonstrate an increase in serum IGF-I if acromegaly is present. The increase in serum IGF-I typically coincides with development and growth of the pituitary somatotropic adenoma. It is important to always interpret IGF-I results in conjunction with the history, physical examination findings, results of routine blood and urine tests, and severity of insulin resistance. A computed tomography (CT) or magnetic resonance imaging (MRI) scan is recommended to confirm the existence of a pituitary macrotumor if the clinical picture supports acromegaly and the serum IGF-I concentration is increased.

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9

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Gastrointestinal, Pancreatic, and Hepatic Disorders

- **Differentiation of Expectoration, Regurgitation, and Vomiting**
  - Expectoration
  - Regurgitation
  - Vomiting
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    - Extraalimentary Tract Disease
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    - Gastritis, Enteritis, and Colitis
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  - Icterus
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- **Aspartate Transferase**
- **Serum Alkaline Phosphatase**
- **Gamma-Glutamyl Transpeptidase**
- **Lactic Dehydrogenase**
- **Sulfobromophthalein Retention**
- **Indocyanine Green**
- **Bile Acids**
- **Ammonia and Ammonia Tolerance Testing (ATT)**
- **Cholesterol**
- **Weight Loss or Anorexia of Unknown Cause**
- **Abdominal Pain**

Gastrointestinal (GI) problems (e.g., vomiting, diarrhea, weight loss, anorexia, icterus, hepatomegaly, abnormal behavior associated with eating, abdominal pain) typically necessitate laboratory testing. Dysphagia, regurgitation, ptyalism, halitosis, constipation, mucoid stools, hematochezia, and melena are best approached initially by other means (e.g., physical examination, radiology, endoscopy, surgical biopsy).

DIFFERENTIATION OF EXPECTORATION, REGURGITATION, AND VOMITING

Whenever fluid, mucus, foam, food, or blood is expelled from the mouth, one must determine whether vomiting, regurgitation, gagging, or expectoration is occurring. The history sometimes allows differentiation.

Expectoration

Expectoration is the coughing up of material from the lungs or major airways. The material typically is frothy mucus or red blood; bile is absent. The characteristic sequence of coughing followed by oral expulsion must be determined from the history. Regurgitation and vomiting typically occur without simultaneous coughing, although regurgitation is often accompanied by tracheitis and aspiration pneumonia. Of the three, expectoration should be the easiest to identify.

Regurgitation

Regurgitation is due to oral, pharyngeal, or esophageal dysfunction and is typically characterized as a relatively passive expulsion of esophageal contents. Gagging is the expulsion of oral or pharyngeal material and may be associated with disorders causing dysphagia (i.e., difficult swallowing) or regurgitation. The relatively minor abdominal contractions associated with gagging can often be differentiated from the vigorous abdominal contractions that occur with vomiting. Regurgitation may follow seconds to hours after eating or drinking. If only saliva is regurgitated, eating may not have occurred for hours or even days before the act. Regurgitated food material is undigested and sometimes has a tubular form conforming to the shape of the esophageal lumen. Most clients cannot reliably distinguish undigested from digested food. Regurgitated material that has remained in the esophagus for long time periods often becomes macerated and odoriferous and is mixed with saliva and mucus. If blood is present it is usually undigested (i.e., bright red), whereas blood originating from the stomach is usually partially digested by gastric acid and has a "coffee ground" appearance readily distinguishing it from the undigested form.

It is sometimes difficult to differentiate vomiting from regurgitation via history, and in some patients the processes are concurrent. Vomiting may cause secondary esophagitis and subsequent regurgitation, or a patient with longstanding esophageal disease may develop another concurrent disorder causing vomiting. It is therefore important to clarify the chronologic order of specific signs. Finally, some patients with signs suggesting regurgitation are vomiting. To aid in differentiation, one may attempt to observe the act of expulsion by feeding the patient, although this is very unreliable ("watched" regurgitating patients often do not regurgitate). Watching the patient eat may be helpful, because pharyngeal dysphagia is usually obvious and suggests oropharyngeal disease. Some patients with pharyngeal dysphagia also have concurrent esophageal dysfunction. Contrast radiographs of the pharynx and esophagus usually differentiate the two disorders.

Regurgitation is usually best evaluated by history, physical examination, plain and contrast radiographs, or esophagoscopy (Figure 9-1). Contrast radiographs should use barium instead of iodide contrast agents unless esophageal rupture is strongly suspected. The main purpose of a contrast esophagram is to distinguish esophageal motility abnormalities from lesions such as an obstruction, diverticulum, or fistula. Some drugs (e.g., xylazine) commonly used for restraint cause esophageal paralysis, making the radiographs potentially misleading. Esophagoscopy may not diagnose esophageal muscular weakness but is effective for sampling mass lesions, differentiating intramural from extramural obstruction, identifying esophagitis, detecting diverticula, and removing foreign objects. Patients with acquired esophageal weakness (i.e., megaesophagus) should be evaluated for myopathies, neuropathies, and myasthenia gravis (generalized or localized to the esophagus). Occasionally, hypoadrenocorticism, hyperkalemia, lead poisoning, *Spirocerca lupi*, and selected central nervous system (CNS) disorders (e.g., distemper, hydrocephalus) may be responsible. Generalized or localized

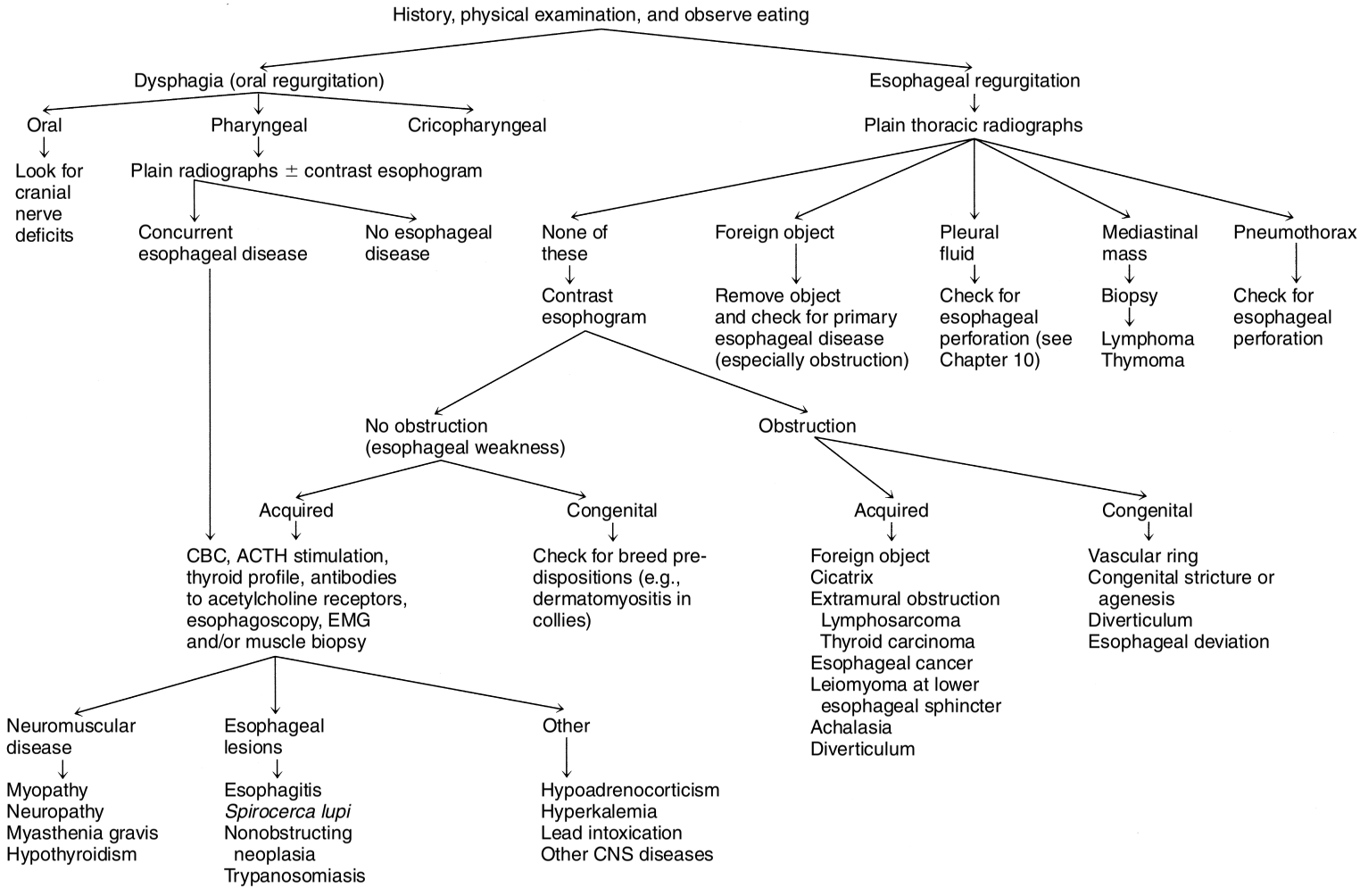

FIGURE 9-1. Diagnostic approach to chronic regurgitation in dogs and cats. *ACTH,* Adrenocorticotropic hormone; *CBC,* complete blood count; *EMG,* electromyogram; *CNS,* central nervous system.

myopathies and neuropathies have several causes, such as trauma, dermatomyositis, thymoma, botulism, tick paralysis, hypothyroidism, hyperadrenocorticism, systemic lupus erythematosus, nutritional factors, toxoplasmosis, and trypanosomiasis. Dysautonomia has been recognized in dogs and cats and causes generalized dysfunction of the autonomic nervous system, including regulation of esophageal motility. Hypothyroidism and systemic lupus erythematosus may exist without obvious clinical signs. It is important to detect these underlying disorders so that one may treat the cause rather than just the symptoms. It is also wise to evaluate patients with unexpected esophageal foreign objects (e.g., a relatively small bolus of food) for partial obstructions (e.g., subclinical vascular ring anomaly, stricture).

Vomiting

Vomiting is a reflex act originating in the CNS that can be stimulated by various conditions. One must consider primary GI disease and non-GI disorders as causes of vomiting. Examples of non-GI disorders include metabolic, inflammatory, and toxic conditions. Many vomiting patients are probably vomiting from non-GI instead of primary GI problems.

Vomiting is classically characterized by prodromal nausea (i.e., salivation, licking of lips) followed by retching or forceful abdominal contractions. Vomiting may occur any time after eating or drinking (seconds to hours). A patient may vomit food, water, fresh blood, or mucus that is indistinguishable from regurgitated material. Bile, partially digested blood (i.e., "coffee grounds"), or expelled material with a pH of five or less confirms that vomiting is occurring. Vomited duodenal contents may have a pH greater than or equal to six and are usually positive for bile. A urine dipstick with a pH indicator is useful in making pH determinations.

Clinically, vomiting patients are best divided into those with acute (<2 weeks) versus those with chronic (>2 weeks) vomiting. The most common categories of causes for each are listed in Tables 9-1 and 9-2. Acute vomiting often spontaneously resolves if the patient is supported by fluid, electrolyte, and acid-base therapy. A thorough history and physical examination are indicated first. Laboratory evaluation (including electrolytes and acid-base evaluations) or imaging should be considered next if the disease is severe. If vomiting persists, is progressive, or is attended by other clinical signs (e.g., polyuria-polydipsia [pupd], weight loss, icterus, painful abdomen, ascites, weakness, hematemesis), additional testing is also indicated (Figure 9-2).

TABLE 9-1. Major Causes of Acute Vomiting in Dogs and Cats

Eating Inappropriate or Spoiled Foods
Motion Sickness
Postoperative Nausea
Acute Gastritis-Enteritis (various viral or bacterial agents or toxins)
Parvoviral enteritis (dogs and cats)
Hemorrhagic gastroenteritis
Parasites
Gastrointestinal (GI) Obstruction
Obstructing foreign body
Linear foreign body
Intussusception
Dietary Indiscretion
Overeating
Eating inappropriate or spoiled foods
Acute Pancreatitis
Drug Administration
Adriamycin
Chloramphenicol
Cisplatin
Cyclophosphamide
Digitalis
Erythromycin
Narcotics
Nitrofurantoin
Tetracycline
Theophylline
Xylazine
Intoxications
Ethylene glycol
Herbicides
Organophosphates
Strychnine

Diet and Parasites

Diet and parasites commonly cause acute and chronic vomiting; hence, dietary change (to a bland or hypoallergenic diet), fecal examination, and broad-spectrum anthelmintic therapy (e.g., fenbendazole, pyrantel) are reasonable initial choices in nonobstructed patients. Continued vomiting is an indication for laboratory tests or imaging.

Obstruction

Gastric or intestinal obstruction does not require clinicopathologic testing for diagnosis. A complete blood count (CBC) may suggest sepsis, disseminated intravascular coagulation (DIC), or severe blood loss. Renal function,

TABLE 9-2. Major Causes of Chronic Vomiting in Dogs and Cats

Obstructive Disease
Foreign objects *(especially common)*
Intussusception
Neoplasia (gastric or intestinal)
Pyloric stenosis
Gastric antral mucosal hyperplasia
Inflammatory infiltrates (gastric or intestinal)
Chronic partial gastric volvulus
Idiopathic hypomotility of stomach/intestines (physiologic obstruction) *(rare)*
Congenital structural abnormalities *(rare)*
Inflammatory Disease
Inflammatory bowel disease *(common)*
Pancreatitis *(common)*
Chronic gastritis
Gastrointestinal (GI) ulceration/erosion
Peritonitis (sterile or septic)
Pharyngitis (caused by upper respiratory virus in cats)
Parasites (e.g., *Physaloptera*)
Systemic (extraalimentary tract diseases stimulating the chemoreceptor trigger zone and/or vagal afferents) (*common*)
Hepatic disease/insufficiency
Hypoadrenocorticism
Diabetic ketoacidosis
Uremia
Hypercalcemia
Cholecystitis
Pyometra
Miscellaneous Causes
Feline hyperthyroidism *(common)*
Feline heartworm disease *(questionable)*
Central nervous system (CNS) disease (e.g., limbic epilepsy, tumor, encephalitis, or increased intracranial pressure) *(rare)*
Psychotic or behavioral changes *(rare)*
Early congestive heart failure *(questionable)*

electrolyte, and acid-base evaluations are recommended before anesthesia. One cannot reliably predict changes in these parameters even when the site of obstruction is known. Gastric vomiting sometimes causes hypokalemic, hypochloremic metabolic alkalosis with aciduria. These changes generally occur secondary to persistent and profuse vomiting, gastric outflow obstruction, or high duodenal obstruction. Most patients with gastric vomiting are not alkalotic. Insignificant acid-base changes or metabolic acidosis due to dehydration with resultant lactic acidosis is perhaps more common. Intestinal obstruction may cause acidosis as the result of loss of pancreatic bicarbonate, although some patients have a normal blood pH or a metabolic alkalosis if the obstruction is high.

Abdominal palpation and imaging are the best initial diagnostic tests. In otherwise occult cases, contrast radiographs may be necessary. Barium is preferred over iodide compounds unless intestinal rupture is strongly suspected. Barium leakage causes peritonitis and requires vigorous abdominal lavage at the time of surgery (see Chapter 10).

Extraalimentary Tract Disease

A serum chemistry profile should be obtained to help rule out hepatic disease (alanine aminotransferase [ALT], serum alkaline phosphatase [SAP], blood urea nitrogen [BUN], and albumin), hypoadrenocorticism (sodium and potassium), hypercalcemia (calcium and albumin), uremia (creatinine, BUN, and urinalysis), and diabetic ketoacidosis (glucose and urinalysis). Very young patients (those < 12 to 14 weeks of age) should undergo blood glucose monitoring to avoid secondary hypoglycemia. More precise testing is occasionally required to diagnose these disorders (e.g., serum bile acids for hepatic insufficiency, adrenocorticotropic hormone [ACTH]-stimulation test for hypocortisolemia). Other tests to consider are serum gastrin for gastrinoma, and serum thyroxine for feline hyperthyroidism.

Pancreatitis

Acute pancreatitis is recognized commonly. Predisposing causes in dogs include hyperlipidemia, fatty meals, or obesity. Pancreatitis can occur in any dog, but middle-aged obese female dogs, schnauzers, and Yorkshire terriers seem to be most commonly affected. Vomiting may or may not be associated with eating, abdominal pain, fasting hyperlipidemia, bloody diarrhea, and rarely, diffuse subcutaneous fat necrosis. On radiographic examination, a mass or indistinctness (as the result of localized peritonitis) may be visible in the cranial right abdominal quadrant. Serum amylase and lipase activities can be measured, but false-negative and false-positive results are common, necessitating reliance on other findings. Leukocytosis with or without a left shift and with or without WBC toxicity (as the result of the sterile inflammation) and increased ALT and SAP concentrations (as the result of the proximity of the pancreas to the liver and obstruction of the biliary duct) are common. The latter occasionally causes extrahepatic biliary tract obstruction and subsequent icterus. Mild to moderate hypocalcemia may occur. Abdominal ultrasonography seems to be an excellent test for canine pancreatitis, and it usually reveals abnormalities in the

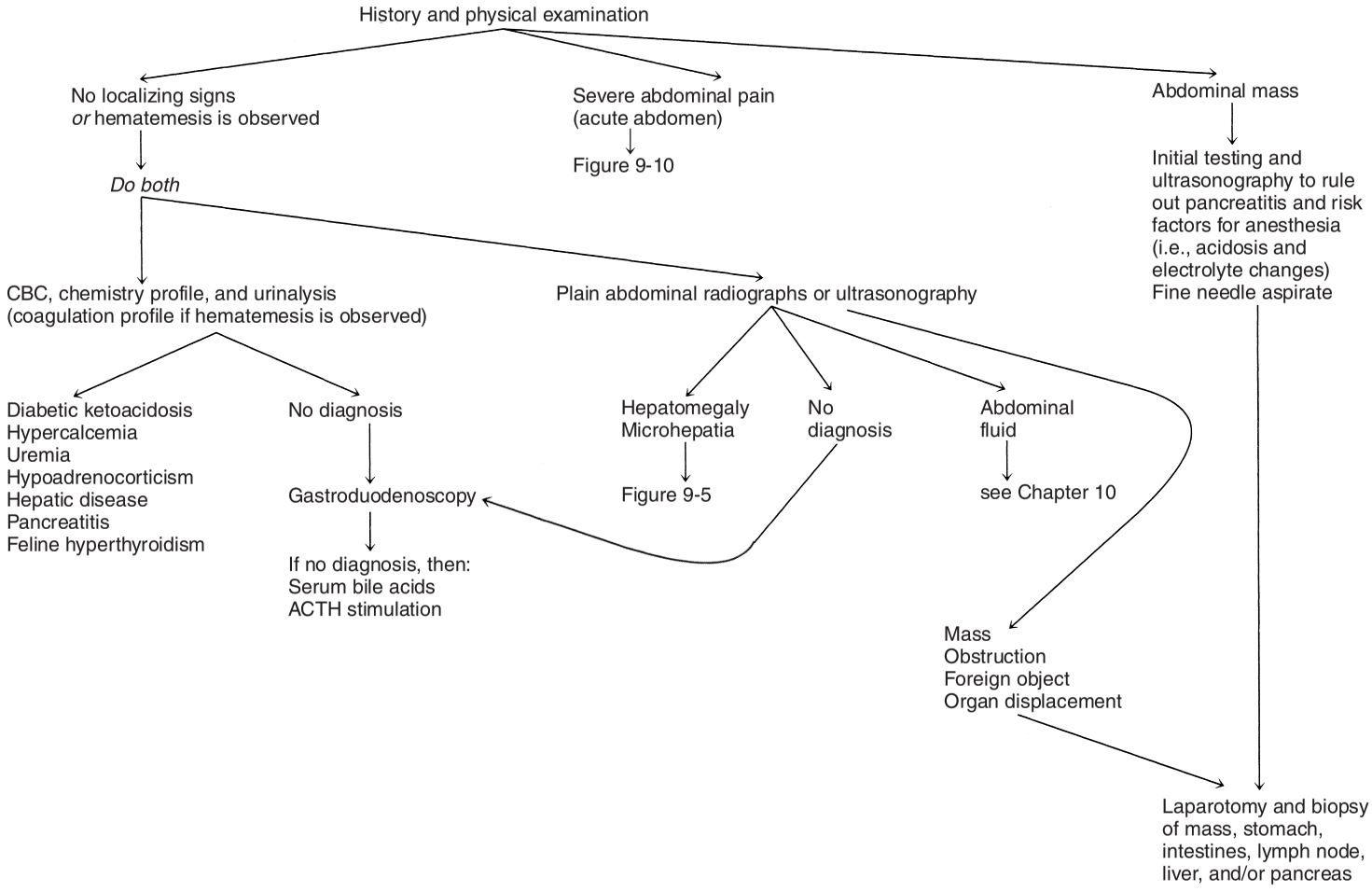

FIGURE 9-2. Diagnostic approach to chronic vomiting in a dog or cat that has been unresponsive to dietary change and anthelmintic therapy. *ACTH,* Adrenocorticotropic hormone; *CBC,* complete blood count.

pancreatic region. If a pancreatic mass is discovered during surgery, it must be biopsied; this is because chronic pancreatitis is grossly indistinguishable from pancreatic neoplasia, and both may be associated with normal or increased serum amylase and lipase values. Recently an assay for immunoreactive canine pancreatic lipase has been developed and validated (GI Laboratory at Texas A&M University); this test seems to hold promise for being a sensitive, specific test for acute pancreatitis.

Once considered rare in cats, pancreatitis is being recognized with increasing frequency in cats. Feline pancreatitis is more difficult to diagnose. Chronic pancreatitis is not uncommon in older cats, occurring in conjunction with cholangiohepatitis and sometimes with inflammatory bowel disease. The presence of the three diseases together has been referred to as "triaditis syndrome." Vomiting is not as prominent as in dogs. Amylase and lipase values are usually in the normal reference range; feline trypsin-like immunoreactivity (fTLI) concentrations are increased in some patients. Abdominal ultrasonography may be useful if an obvious abnormality is found, but the sensitivity of ultrasonography is uncertain. A pancreatic biopsy may be required for a definitive diagnosis. Feline pancreatitis occasionally is due to toxoplasmosis or to feline infectious peritonitis (FIP) (see Chapter 15).

Gastritis, Enteritis, and Colitis

Chronic enteritis, colitis, or gastritis can cause various degrees of vomiting and may require mucosal biopsy for diagnosis. Abdominal imaging may delineate infiltrative or inflammatory intestinal patterns. If gastritis or enteritis is suspected or if the other major causes of chronic vomiting have been ruled out, gastric and intestinal mucosal biopsies via endoscopy or laparotomy are indicated. Inflammatory bowel disease is a significant cause of feline chronic vomiting. Duodenitis is also a significant cause of vomiting without diarrhea in dogs; therefore, both gastric and intestinal biopsies should be performed. Finally, because 10% to 20% of patients with colitis vomit, it is useful to perform endoscopy routinely on both the upper and lower intestinal tracts in patients (especially cats) with chronic vomiting. It is critical that mucosal tissue samples be taken and handled properly to avoid artifacts, which can render them nondiagnostic.

Hematemesis

Hematemesis is the vomiting of blood. It suggests gastric ulceration. The character of the vomitus may be either bright-red blood or digested blood that resembles coffee grounds. Administration of nonsteroidal anti-inflammatory drugs (especially concurrently with corticosteroids) is a major reason for canine ulceration. Renal and hepatic failure, mast cell tumor, shock with poor mucosal perfusion, and coagulopathy must be considered. After these have been ruled out, endoscopy is indicated and allows diagnosis of ulceration (especially because of a foreign object, inflammatory disease, or neoplasia). Alternatively, one may treat symptomatically for ulceration; however, such treatment may allow progression of underlying disease.

Abdominal Inflammation

Septic or nonseptic peritonitis (or inflammation of any abdominal organ) may cause vomiting. Abdominocentesis or abdominal lavage (see Chapter 10) may be needed, especially if physical examination or abdominal imaging suggests abdominal fluid. Occult cases may require exploratory surgery for diagnosis.

Gastrinoma

Gastrinoma (e.g., Zollinger-Ellison syndrome) is a gastrin-secreting tumor of the pancreatic islet cells; it increases gastric acid production and produces duodenal ulceration. Gastrinoma is rare but has been diagnosed more commonly since the advent of reliable serum gastrin assays. No other typical, unique clinicopathologic tests exist that suggest this disease. Any chronically vomiting middle-aged or older dog with weight loss or diarrhea is a reasonable suspect. Duodenal ulceration and reflux esophagitis are common. Resting gastrin concentrations are usually increased, but in rare cases one must measure gastrin concentrations after administering food or secretin.

AMYLASE

Controversial Indications • Patients (especially obese) with vomiting, abdominal pain, nonseptic inflammatory abdominal exudate, icterus, or a prior history of pancreatitis.

Disadvantages • Poor sensitivity and poor specificity. Serum amylase activity does not correlate with the severity of pancreatitis.

Analysis • Measured in serum, heparinized plasma, or body fluid by spectrophotometric methods via amyloclastic, saccharogenic, and chromogenic techniques. Turbidimetric, nephelometric, and "dry reagent" methods may also be used.

NOTE: Different methods can give substantially different values. Some saccharogenic methods are affected by normal canine serum maltase concentrations and should not be used in dogs. Serum amylase activity is stable at room temperature for up to 7 days and at 4° C for as long as 1 month.

Normal Values • As with other enzymes, these vary among laboratories, depending on the technique and units used.

Danger Values • None.

Artifacts • See Introduction to Serum Chemistries.

Drug Therapy That May Cause Hyperamylasemia • Some drugs may occasionally cause pancreatitis (Table 9-3). Corticosteroids do not reliably increase serum amylase concentrations.

Causes of Hypoamylasemia • Insignificant. This finding does not support a diagnosis of pancreatic insufficiency.

TABLE 9-3. Drugs That May Cause Acute Pancreatitis

Asparaginase
Azathioprine
Calcium
Estrogens
Furosemide
Glucocorticoids (especially dexamethasone)
Isoniazid
Metronidazole
Potassium bromide (this is a reported but unproven association)
Salicylazosulfapyridine (Azulfidine)
Sulfonamides
Tetracycline
Thiazide diuretics

NOTE: These drugs do not reliably cause pancreatitis, and a history of administration of one of these drugs plus signs of pancreatitis cannot be assumed to be cause and effect. A patient with acute pancreatitis that is receiving one of these drugs, however, should undergo drug withdrawal, if possible.

Causes of Hyperamylasemia • Decreased glomerular filtration (i.e., azotemia) and pancreatitis are two causes. Hyperamylasemia as the result of renal dysfunction usually is less than two to three times the upper limit of normal. Patients with pancreatitis may have normal to markedly increased values. Intestinal disease, ruptured intestines, and hepatic disease *have been suspected* of causing increased serum amylase because of amylase present in these tissues. Serum amylase level is an unreliable indicator of pancreatitis in cats. Hyperamylasemia in a vomiting or anorexic animal is an indication to search for pancreatitis by CBC, serum chemistry profile (including ALT and SAP), abdominal imaging, serum trypsin-like immunoreactivity (TLI), or a combination thereof.

Causes of Increased Fluid Amylase • When abdominal fluid amylase is greater than serum amylase, a nonseptic exudate caused by pancreatic disease is possible. Bowel rupture may also be possible.

LIPASE

Controversial Indications • Same as for amylase.

Disadvantages • Questionable sensitivity and specificity; some dogs with duodenal foreign objects, chronic gastritis, and abdominal carcinomas have very increased serum lipase activity without evidence of pancreatitis. Serum lipase activity does not correlate with the severity of pancreatitis.

Analysis • Measured in serum or body fluids via dry reagent analysis. Turbidimetric and titrimetric techniques are rarely used.

Normal Values • As for other enzymes, these vary from laboratory to laboratory, depending on the technique and units used.

Danger Values • None.

Artifacts • See Introduction to Serum Chemistries.

Drug Therapy That May Cause Hyperlipasemia • Same as for amylase (see Table 9-3) plus heparin. Corticosteroids (dexamethasone) may increase serum lipase activity up to fivefold over baseline without

histologic evidence of acute pancreatitis; however, the lipase activity is usually only slightly greater than the reference range.

Causes of Hypolipasemia • Not significant. This finding does not support a diagnosis of pancreatic insufficiency.

Causes of Hyperlipasemia • These are similar to the causes of hyperamylasemia. Renal dysfunction increases serum lipase, usually less than two to three times normal, although it may rarely be more than four times normal. Not all patients with acute pancreatitis have increased serum lipase, and the increase in serum lipase activity is not proportional to the severity of the pancreatitis. Extremely increased lipase values have been associated with pancreatic carcinomas. Abdominal ultrasonography and increases in serum fTLI appear to be more useful than serum amylase or lipase in the diagnosis of feline pancreatitis. In addition to serum TLI, trypsin-activating peptide (TAP) and phospholipase A_2 have been investigated as tests for pancreatitis in dogs; however, none have become accepted as valued clinical tests.

CANINE IMMUNOREACTIVE PANCREATIC LIPASE

Common Indications • Patients (especially obese) with vomiting, abdominal pain, nonseptic inflammatory abdominal exudate, icterus, or a prior history of pancreatitis.

Advantages • Appears to be sensitive and specific and only requires a serum sample.

Disadvantages • Currently the only laboratory offering this test for dogs is GI Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4474.

Analysis • Measured in serum by enzyme-linked immunosorbent assay (ELISA). See Appendix I for availability.

Normal Values • 2.2 to 102.1 µg/L.

Artifacts • Uncertain.

Causes of Decreased Values • Exocrine pancreatic insufficiency (EPI) or isolated pancreatic lipase deficiency. However, slightly more overlap exists between normal dogs and dogs with EPI than for serum TLI concentration. Thus serum TLI remains the test of choice for EPI.

Causes of Increased Values • Pancreatic inflammation is currently the only recognized cause of an increase. Further experience with this test may change the indications and interpretation of this test.

GASTRIN

Occasional Indications • Chronic vomiting, diarrhea, weight loss, suspected gastrinoma, or gastric or duodenal ulceration of unknown cause. This test is usually not requested until more common diseases have been ruled out.

Advantages • Detects otherwise occult gastrinomas.

Disadvantages • Requires radioimmunoassay (RIA) method (long turnaround time).

Analysis • Measured in serum by RIA. Serum should be frozen until assayed. See Appendix I for availability.

Normal Values • Dogs, depends on laboratory (the assay must be validated for dogs); cats, not established.

Conversion of pg/ml to ng/L: multiply pg/ml × 1.0 = ng/L.

Artifacts • Falsely decreased: hormone degradation as the result of storage for several days at temperatures above freezing.

Drug Therapy That May Increase Gastrin • Antacids including H_2 receptor antagonist drugs, and proton pump inhibitors.

Causes of Hypogastrinemia • Not significant.

Causes of Hypergastrinemia • Atrophic gastritis (uncommon), antral G-cell hyperplasia (rare), short bowel syndrome, hyperparathyroidism, ulcers, gastric outlet obstruction, renal failure, and gastrinoma are the main causes. The last four are the most common. Hepatic insufficiency does not appear to directly increase serum gastrin concentrations. If gastrinoma is suspected in a patient that has a normal or equivocal serum gastrin concentration, secretin or calcium stimulation tests

may be performed. A rise in the serum gastrin concentration after giving either of these drugs suggests a gastrinoma.

ACUTE DIARRHEA

Patients with diarrhea are best classified into those with acute (<2 to 3 weeks) versus those with chronic (>2 to 3 weeks) diarrhea. Acute diarrhea (Table 9-4) is usually self-limiting, although some conditions may be severe and cause mortality, such as acute hemorrhagic gastroenteritis, parvoviral disease, parasites (e.g., hookworms), or intoxication. History should explore the possibility of recent dietary change or exposure to infectious agents. Diet, bacteria, viruses, and parasites are the major identifiable causes of acute diarrhea in dogs and cats. Because intestinal parasites may contribute to any diarrheic state, multiple fecal examinations (direct and flotation) are warranted in all diarrheic patients. Giardiasis may be particularly occult and require special diagnostic techniques (see Fecal *Giardia* Detection).

Feeding with bland or hypoallergenic diets may be diagnostic and therapeutic. Depressed, weak, and dehydrated patients should undergo electrolyte and acid-base evaluations to aid in selecting fluid replacement therapy. All patients less than 12 to 14 weeks of age and those that are emaciated or weighing less than 5 pounds should undergo blood glucose monitoring to detect secondary hypoglycemia. Febrile or depressed patients should undergo CBC analysis so that sepsis or transmural inflammation can be detected. To identify the cause of acute diarrhea that is not the result of diet or parasites (such as that occurring in kennels, pet stores, shelters, and households where more than one member has diarrhea), fecal cultures for *Salmonella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, verotoxin-positive *Escherichia coli*, and other pathogens plus viral identification methods (i.e., ELISA, electron microscopy) or toxin identification methods (i.e., ELISA for *Clostridium perfringen*s or *Clostridium difficile* toxins) or both may be used.

Not all patients with canine parvoviral diarrhea are severely ill, have identifiable leukopenia, have diarrhea, or have a fever. Leukopenia may persist as briefly as 24 to 36 hours and can easily be missed if a CBC is not performed during that period. Other diseases causing severe sepsis (i.e., perforating linear foreign body with peritonitis or overwhelming salmonellosis) can cause leukopenia indistinguishable from that of canine parvoviral diarrhea. Routinely used vaccination schedules do not necessarily guarantee protection against canine parvovirus. Finally, fecal shedding of viral particles may not occur for 1-3 days after signs begin and decreases rapidly with time. In-house ELISA tests for parvovirus are performed on the feces and appear to be accurate in identifying the parvoviral antigen, but testing may be negative if done too early or too late. The test result should be strongly positive within 3 days of the onset of clinical signs and remain positive for several days. A recent vaccination may result in a weakly positive fecal ELISA.

TABLE 9-4. Major Categories of Causes of Acute Diarrhea in Dogs and Cats

Intestinal Parasites
Hookworms
Roundworms
Whipworms
Coccidia
Giardia (sometimes difficult to diagnose)
Strongyloides
Tritrichomonas
Dietary Problems
Poor-quality food/food poisoning
Sudden dietary change (especially young animals)
Food intolerance/allergy
Acute Viral or Bacterial Enteritis
Parvovirus (canine and feline)
Coronavirus (canine and feline)
Clostridium perfringens
Campylobacteriosis
Salmonellosis
Escherichia coli (verotoxin-producing strains)
Intussusception
Intoxication
Garbage
Food poisoning
Heavy metal
Organophosphate
Hemorrhagic Gastroenteritis

CHRONIC DIARRHEA

Chronic diarrhea should first be defined as either small intestinal or large intestinal in origin, preferably by using the history and physical examination (Table 9-5). Occasionally, large and small intestines are concurrently involved. Patients with chronic diarrhea in which clinical disease is not severe are often treated with therapeutic trials before aggressive diagnostics are instituted. All patients should

TABLE 9-5. Differentiation of Chronic Small Intestinal from Chronic Large Intestinal Diarrhea

	SMALL INTESTINAL DIARRHEA	LARGE INTESTINAL DIARRHEA
Weight loss (most important criteria)	Expected	Uncommon except with histoplasmosis, pythiosis, or cancer
Polyphagia	Often present	Uncommon
Vomiting	May occur	Occurs in 10% to 20% of patients
Volume of feces	May be normal or larger than normal	May be normal or smaller than normal
Frequency of defecation	Normal to slightly increased	Normal to markedly increased, may have many small defecations per bowel movement
Slate-gray feces (steatorrhea)	Occasionally	No
Hematochezia	No	Sometimes present
Melena	Sometimes	No
Mucoid stools	Rare (unless ileum is diseased)	Often present
Tenesmus/dyschezia	Rarely present	Sometimes present

undergo at least three fecal examinations at 48-hour intervals. If these tests are negative, it is still acceptable to treat empirically for *Giardia* infection and whipworms before aggressive diagnostics are begun. Giardiasis may be particularly difficult to diagnose (see Fecal *Giardia* Detection) and medically manage. Adverse food reactions also cause chronic diarrhea. Dietary intolerances are a reaction to a particular substance in the diet, whereas true food allergies are immunologic reactions to specific antigens. Food reactions are common, especially in cats. Dietary food trials are indicated in suspected cases. Failing to respond to empiric therapy indicates the need for further diagnostics.

Large Intestinal Diarrhea

Once parasitism, dietary-responsive disease, and clostridial colitis are eliminated, additional simple diagnostic steps, such as rectal mucosal scrapings (not swabs) with cytologic examination (see Color Plate 3B) or fecal culture for *C. jejuni, Salmonella* spp., *Y. enterocolitica,* verotoxin-positive *E. coli*, or a combination of these might be appropriate. Persistent large intestinal disease is usually an indication for colonoscopy plus biopsy, especially if the animal has hypoalbuminemia or has lost weight. Rigid colonoscopy of the descending colon is adequate for diagnosis in most cases. Flexible endoscopy allows access to the descending, transverse, and ascending colon; ileocolic valve; cecum; and ileum. If flexible endoscopy is unavailable, abdominal ultrasonography or a barium enema may reveal lesions in areas not accessible with rigid endoscopy.

Small Intestinal Disease

Chronic and severe small intestinal diarrhea necessitates differentiation of maldigestion, protein-losing enteropathy (PLE), and malabsorptive disease without protein loss (Figure 9-3). Weight loss and diarrhea are usually present, but some patients only have weight loss.

Maldigestion

Maldigestion resulting from bile acid insufficiency as the result of biliary obstruction is rare. Intestinal lactase deficiency is uncommon, but a lactose-free diet may be tried in selected patients (especially cats). EPI is the most common cause of canine maldigestion but is rare in cats. Differentiation of EPI from malabsorptive disease is important. The diagnosis is often overlooked in afflicted dogs or may inappropriately be made in patients without the malady. Clinical trials using pancreatic enzyme preparations are commonly used to diagnose EPI. Unfortunately this method is unreliable. Powdered enzyme is often superior to tablet formulations, and some enzyme preparations are clearly superior to others. Even when appropriate enzymes are administered, some dogs with EPI also require a low-fat diet, antacid therapy (rare), or treatment for concurrent antibiotic-responsive enteropathy (ARE) (common) before the enzyme replacement therapy becomes effective. Too often, failure of the initial enzyme replacement therapy leads to unnecessary tests (i.e., exploratory laparotomy), because the correct diagnosis of EPI was incorrectly eliminated.

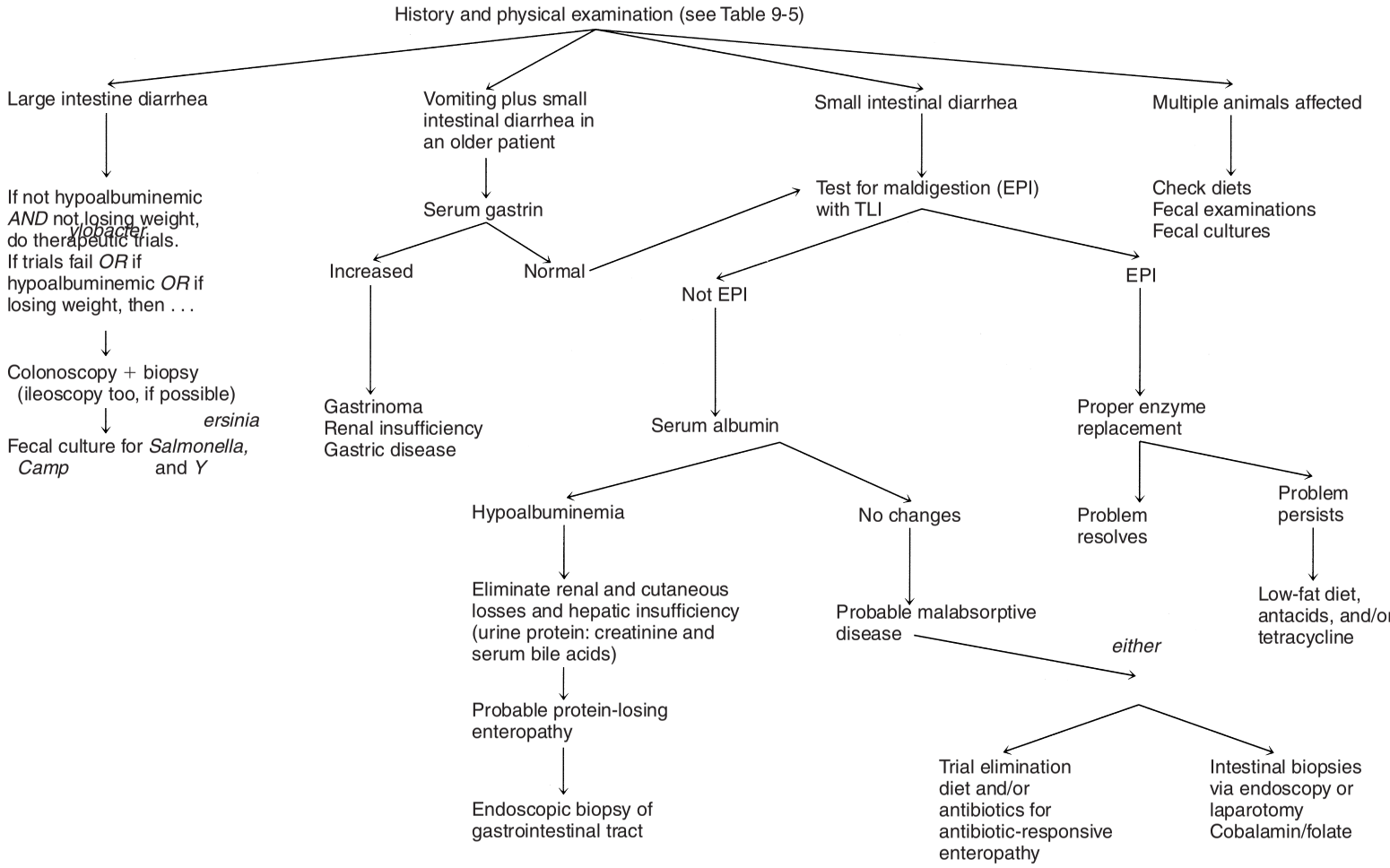

FIGURE 9-3. Diagnostic approach to chronic diarrhea in dogs and cats in which multiple fecal examination results are negative and empiric anthelmintic, antiprotozoal, and dietary therapy do not resolve the diarrhea. *EPI,* Exocrine pancreatic insufficiency; *TLI,* trypsin-like immunoreactivity.

No consistent hematologic or serum chemistry profile changes are seen, and the serum amylase and lipase values are usually normal. Undigested fats are often found in the feces; however, this is inconsistent. The fat absorption test is inexpensive but can yield false results. The TLI assay is the best test for canine EPI, and the fTLI assay is the best test for feline EPI. It is important to note that the tests are species specific. Measurement of trypsin proteolytic activity also is an accurate means of diagnosing feline EPI; however, it is more cumbersome and has limited availability.

Malabsorptive Disease Without Protein Loss

Once maldigestion has been accurately ruled out, malabsorption becomes the most likely diagnosis in diarrheic animals with weight loss. One must then decide whether to perform diagnostic therapeutic trials or diagnostic tests. A definitive diagnosis usually necessitates intestinal biopsy. Patients that are critically ill (i.e., are emaciated or have serum albumin <2.2 g/dl) usually should next undergo abdominal ultrasonography and intestinal biopsies (preferably via endoscopy). Patients that are not critically ill may be managed first using therapeutic trials. Therapeutic trials may be chosen more rationally with the aid of minimal laboratory data such as a CBC, biochemical profile, and fecal examinations. The two major therapeutic trials are (1) food trials for dietary intolerance and (2) antibiotic trials for ARE.

ARE (previously called "small intestinal bacterial overgrowth") may exist by itself or it may coexist with another GI malady. ARE may prevent therapy aimed at the underlying problem from resolving clinical signs. No consistent CBC or serum chemistry profile changes are seen in this syndrome. Fecal culture is not informative, and intestinal biopsy is seldom diagnostic. A barium contrast study may identify a segmental lesion or partial obstruction responsible for secondary ARE. Quantitated culture of duodenal or proximal jejunal fluid for aerobes and anaerobes is difficult to interpret, because clinically normal dogs may have as many or more bacteria than clinically affected dogs. Finding an increased concentration of unconjugated serum bile acids is believed to be supportive of ARE. Bacteria can deconjugate serum bile acids in the intestinal lumen, and these bile acids can be absorbed by the jejunum. However, the test is limited in its availability. Serum vitamin B_{12} and folate concentrations have been used as screening procedures for ARE once EPI has been ruled out. Some patients with ARE have normal serum vitamin B_{12} and folate concentrations, however. Response to empiric antibiotic therapy supports the diagnosis. Signs secondary to ARE usually respond to appropriate antibiotic therapy (e.g., tetracycline, tylosin, ampicillin, metronidazole) unless irreversible mucosal changes or a primary underlying intestinal disease are seen.

Dietary intolerance is relatively common, and hypoallergenic diets (e.g., fish and potato, turkey and potato, tofu and beans) are reasonable trials. At least 4 weeks (and preferably 6 to 8 weeks) should be allotted for such a dietary trial, during which time absolutely nothing else should be fed (including flavored treats or medications).

If dietary, antibiotic, and repeated anthelmintic and antiprotozoal therapies are ineffective, small intestinal biopsy is probably necessary. Laparotomy, laparoscopy, or endoscopy may be used. In most patients, the stomach, duodenum, ileum, and colon may be endoscopically sampled. Duodenal cytology is helpful in some disorders (e.g., eosinophilic enteritis, purulent enteritis, giardiasis, lymphoma). If laparotomy is performed, multiple representative full-thickness specimens (e.g., stomach, duodenum, jejunum, ileum, mesenteric lymph node) are indicated, because lesions can be sporadic. If endoscopy is performed, multiple specimens (e.g., ≥ 8) from each site are obtained. It is critical that the endoscopist be accomplished and trained in obtaining high-quality tissue samples. Many endoscopic biopsies obtain nondiagnostic samples because of the operator's lack of training in this area.

Protein-Losing Enteropathy

PLEs are often characterized by a decrease in serum concentrations of both serum albumin and globulin, which are lost through the GI tract. PLE is uncommon in cats but seen with some regularity in dogs. Dogs with inflammatory diseases causing hyperglobulinemia and some breeds (e.g., Basenji dogs) may have only hypoalbuminemia. This occurs because the serum globulin concentration is greatly increased, and even though much of this fraction is lost into the intestines, the amount remaining keeps concentrations in the normal range. If red blood cells (RBCs)

are also being lost, iron-deficiency anemia may occur (see Chapter 3).

PLE may be the result of various GI diseases (e.g., hookworms, chronic intussusception, fungal infections, ulcers and erosions), but inflammatory bowel disease, alimentary lymphosarcoma, and lymphangiectasia are the most common causes in adult dogs. Intestinal lymphangiectasia causes severe PLE in dogs (it is not reported in cats) and can produce some of the lowest serum protein levels (serum albumin < 1.0 g/dl) that occur in alimentary disease. Because of the loss of lymph into the intestines, peripheral lymphocyte counts may be decreased; hypocholesterolemia and steatorrhea are common. If hepatic insufficiency and loss from the kidneys and skin have been eliminated in a hypoalbuminemic patient, PLE becomes the major differential diagnosis by process of elimination. If PLE is suspected in a patient that has another potential explanation for its hypoalbuminemia (e.g., renal protein loss or substantive hepatic insufficiency), then detecting abnormally high concentrations of alpha-1 protease inhibitor in the feces supports a diagnosis of GI protein loss. The relatively stable alpha-1 proteinase is resistant to GI degradation and consequently can be measured in the feces. Intestinal biopsy is usually the definitive test. Full-thickness biopsy may risk dehiscence if the serum albumin level is less than 1.5 g/dl; however, serosal patch graft techniques decrease the risk of dehiscence. Gastroduodenoscopy-ileoscopy plus biopsy is safe and often (but not invariably) diagnostic. Occasionally the intestinal lesion is inaccessible via endoscopy. Although not recommended, dietary trial may be used in patients with PLE. An ultra-low–fat diet is reasonable if lymphangiectasia is suspected; however, therapeutic trials with steroids are potentially dangerous and are not recommended without a definitive diagnosis.

FECAL CHARACTER

Mucoid feces should be approached as a large intestinal or a distal small intestinal problem. In dogs and cats that have large bowel disease but no weight loss or hypoalbuminemia, multiple fecal examinations, digital rectal examination, and therapeutic trials (i.e., dietary, antibacterial or anthelmintic [or both]) are often the best initial steps. If these are unsuccessful, then colonileoscopy plus biopsy generally becomes the most useful diagnostic tool. Hematochezia should also be considered as a large bowel problem. Melena signifies swallowed blood from any source, coagulopathy, or gastric and upper intestinal bleeding. Therefore before performing an exploratory laparotomy, one should consider all the possible causes of oral bleeding (e.g., coughing up blood from the respiratory tract, posterior nasal bleeding). Ingestion of bismuth subsalicylate (Pepto-Bismol) or liver can cause feces to appear melenic. Diet and changes in intestinal bacterial flora influence fecal color but do not generally signify disease.

FECAL ENZYME-LINKED IMMUNOSORBENT ASSAY FOR PARVOVIRUS

Occasional Indications • Dogs suspected of having parvoviral enteritis (especially those not displaying classic signs); acute neutropenia of unknown cause.

Advantages • Quick, available, has good sensitivity and specificity if done at the appropriate time (e.g., approximately 1-3 days after onset of clinical signs).

Disadvantages • Dogs with parvoviral enteritis can have negative reactions, especially early in the course.

Analysis • Fresh feces, preferably taken from a dog that has begun to show signs in the last 24 to 36 hours, are used according to kit instructions (see Chapter 15). The instructions must be carefully followed or false results might be obtained.

Normal Values • Dogs should not have parvoviral antigen in feces.

Interpretation • A positive result supports canine parvoviral enteritis. Not all dogs affected with parvoviral enteritis have diarrhea and fever; some show only anorexia, vomiting, or fever. Theoretically, if coproantibody binds all of the antigen in the feces, a false-negative result may occur. If the test is performed too early in the disease, it may yield negative results. With such dogs, one should repeat the test in 36 to 48 hours. Shedding of viral particles decreases after the first week of disease, and a test performed too late in the disease might yield negative results. Modified-live vaccination results in transient fecal shedding

and can give a weak positive fecal ELISA test result (5 to 15 days after vaccination).

FECAL ANALYSIS FOR CLOSTRIDIAL TOXINS

Occasional Indications • Dogs with acute, nosocomial diarrhea or chronic large bowel diarrhea of unknown cause.

Advantages • Relatively easy to perform.

Disadvantages • Might be difficult to interpret results, especially of old fecal samples. Uncertain sensitivity and specificity for *Clostridium perfringens*—associated diarrhea.

Analysis • Fresh or frozen feces used according to the instructions on the test kit. Reversed passive latex agglutination (RPLA) and ELISA (i.e., *Clostridium perfringens* Enterotoxin Test, TechLab, Blacksburg, VA) methods are available for *Clostridium perfringens* enterotoxin. ELISA methodology is available to look for *Clostridium difficil*e toxin A (ImmunoCard Toxin A, Meridan Diagnostics, Cincinnati OH).

Interpretation • Finding *Clostridium perfringens* enterotoxin in feces plus clinical signs of clostridial diarrhea has been considered diagnostic of clostridial colitis. Results from ELISA methodology appear to correlate better with disease than do results from RPLA methodology. However, production of enterotoxin does not appear to be a consistent event (i.e., found in every bowel movement), especially in the later course of disease. In suspected cases with a negative toxin assay, it might be useful to wait and repeat the test again at the onset of recurrence of clinical signs or perform a therapeutic trial with amoxicillin or tylosin.

Fecal spore counts do not correlate well with *Clostridium perfringens* enterotoxin production or with the presence of diarrhea. Examining fecal smears (see Fecal Microscopic Cytology) to look for the presence of spores no longer seems to be an acceptable screening procedure (e.g., clinically normal dogs may have spores in their feces and be positive for enterotoxin by RPLA).

Finding evidence of *Clostridium difficile* toxin A in feces of diarrheic patients seems suggestive of a cause-and-effect relationship, but this is currently being investigated.

FECAL CULTURE

Occasional Indications • Dogs and cats with persistent diarrhea (especially large bowel) of unknown origin, suspected contagious diarrhea, or a suspected infectious cause (e.g., diarrhea with concurrent fever, leukocytosis, neutrophilic fecal cytology, bloody diarrhea). Enteric pathogens include *C. perfringens, Salmonella* spp., *C. jejuni,* verotoxin-positive *E. coli*, *Clostridium perfringens, Clostridium difficile,* and *Y. enterocolitica.*

Disadvantages • Must specify which pathogens to culture, must provide the laboratory with fresh feces or feces submitted in appropriate transport media, and requires a microbiology laboratory familiar with the specific enrichment and isolation techniques for each pathogen for which a culture is attempted. Using culture swabs is not adequate for isolation of most enteric pathogens. Finally, growing a "pathogen" does not mean that it is responsible for clinical signs.

Analysis • Fresh feces must be promptly submitted to the laboratory, and the laboratory must know the specific pathogen(s) sought. To submit old feces or feces that have not been collected or handled properly or to request a "general culture for pathogens" is generally a waste of time and money. It requires laboratories that are properly equipped to culture for enteric pathogens. Culture for *C. perfringens* and *Clostridium difficile* in particular is not usually diagnostically useful.

Interpretation • Small numbers of any of the pathogens listed earlier might be found in normal pets, although *Y. enterocolitica* is particularly uncommon in the United States. Interpretation of the fecal culture must consider the history, physical examination, laboratory data, and sometimes numbers of pathologic organisms (i.e., number of bacterial colony-forming units per gram of feces) found. With diarrhea from any cause, the GI flora may change from a predominately anaerobic to a gram-negative aerobic population.

FECAL FAT

Rare Indications • To detect malabsorption or maldigestion in animals with diarrhea or unexplained weight loss.

Advantages (Semiqualitative Analysis) • Minimal expense, availability, and reasonable accuracy as a screening test.

Disadvantages (Semiqualitative Analysis) • Occasionally misleading results.

Advantages (Quantitative Analysis) • Very sensitive.

Disadvantages (Quantitative Analysis) • Expense, difficulty in collecting and storing feces and in differentiating between the causes of steatorrhea.

Analysis • The clinician performs a semiqualitative analysis for undigested fats by mixing a drop of fresh feces with a drop of Sudan III, heating the slide to a boil, and examining the smear microscopically. The clinician performs analysis for digested fats by mixing 1 drop of fresh feces, 1 drop of 36% acetic acid, and 1 drop of Sudan III. This is put on a microscope slide, heated to boiling, and examined while still warm. In both cases, identifying orange droplets is a positive finding. It is important that the patient has been eating a moderate- to high-fat diet. Feeding low-fat diets to malabsorptive dogs may cause the test result to be negative.

Quantitative analysis is rarely indicated or performed. The technique is described in prior editions.

Normal Values • Semiqualitative: few or no undigested and digested fat globules per high-power field (hpf).

Artifacts • The semiqualitative analysis may have unexplained false-negative and false-positive reactions. Administration of barium sulfate, bismuth, psyllium fiber, mineral oil, or castor oil or feeding a low-fat diet may also confuse semiqualitative analysis.

Drug Therapy That May Alter Measurement of Fat Excretion • Decreased excretion may be caused by medium-chain triglyceride oil supplements (titrimetric analysis). Increased quantitated fat excretion may be caused by azathioprine, orally administered aminoglycosides, and cholestyramine.

Causes of Increased Fecal Fat • Finding several orange globules/hpf, if repeatable on several examinations, is principally caused by malabsorption or maldigestion. It is a reasonable screening test and helps distinguish maldigestion because of EPI (positive for undigested fats) from malabsorption (positive for digested fats). Despite occasional false-positive reactions, strongly positive results for undigested fecal fat in a dog with signs consistent with maldigestion are an indication for more specific tests (e.g., TLI). Questionable results on the semiqualitative test should always be followed by more specific tests. With Sudan staining, fecal fat may not be detectable in some dogs with EPI.

FECAL STARCH

Rare Indications • Chronic diarrhea or weight loss.

Advantages • Low cost and availability.

Disadvantages • Negative and positive results that do not correlate with malabsorption and maldigestion. This test is *not recommended*.

Analysis • Fecal smears are stained with 2% Lugol's iodine. Starch granules show up as dark blue-black granules when viewed microscopically.

Normal Values • Rare (0 to 5) granules/hpf, although this may vary with the diet.

Artifacts • Falsely increased: contamination of feces with food. Unexplained false-positive and false-negative results may also occur.

Drug Therapy That May Affect Fecal Starch • Some diets may have more fecal starch excretion than others.

Causes of Amylorrhea • EPI is most likely, but high-starch diets or conditions causing increased intestinal transit may cause amylorrhea. Amylorrhea in a patient with weight loss or diarrhea is an indication for serum TLI.

FECAL MUSCLE FIBERS

Rare Indications • Chronic, small bowel diarrhea or weight loss that is difficult to diagnose.

Advantages and Disadvantages • Same as for fecal starch. This test is *not recommended*.

Analysis • A fresh fecal smear is stained with 2% Lugol's iodine, new methylene blue (NMB), or Wright's stain.

Normal Values • Dogs, muscle fibers should not be visible; cats, assumed to be similar to dogs.

Therapy That May Alter Fecal Muscle Fiber Determination • Some diets result in more fecal muscle fibers than others. Administration of barium sulfate, mineral oil, magnesium, or bismuth may make fiber identification difficult. A meat-free diet renders this test useless.

Causes of Creatorrhea • EPI is possible, and TLI is indicated.

FECAL PROTEOLYTIC ACTIVITY

Rare Indications • To detect maldigestion in animals with chronic diarrhea or weight loss of unknown cause.

Advantages • Theoretically this test may diagnose EPI in rare patients that have EPI secondary to obstruction of the pancreatic duct or ducts.

Disadvantages • The radiograph film digestion test is useless and should never be used. The most reliable procedure for measuring fecal proteolytic activity is difficult to perform and requires special handling of the feces; it is described in prior editions.

Analysis • Described in prior editions. The fTLI test has replaced measurement of fecal proteolytic activity as the diagnostic test of choice for EPI in cats.

FECAL ALPHA-1 PROTEASE INHIBITOR ACTIVITY

Infrequent Indications • Hypoalbuminemia of uncertain cause or suspected PLE.

Advantages • Specifically indicates the GI tract as the source of protein loss. Alpha-1 protease inhibitor is a plasma protein, which, if leaked into the intestinal lumen, is resistant to GI degradation and hence can be measured in the feces. The amount of alpha-1 protease inhibitor reflects the approximate loss of plasma proteins into the GI tract.

Disadvantages • Limited availability of the test. The magnitude of alpha-1 protease inhibitor in the feces is variable and may not reflect the severity of the disease.

Analysis • Three 1 g fecal samples from three different bowel movements are submitted in tubes provided by the laboratory. It is critically important that three samples (preferably from different days or at least different bowel movements) be submitted, that the feces be collected promptly after defecation, and that the feces not be collected by digitally removing them from the rectum. Samples must be frozen while one awaits shipping and must be shipped on a cold pack. Currently the only laboratory offering this test for dogs is GI Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4474.

Normal Values • 0.23 to 5.67 μg/g feces.

Causes of Abnormalities • Abnormally high values in the feces indicate loss of serum proteins into the alimentary tract and might indicate that PLE is the cause of hypoalbuminemia. Interpretation of the magnitude of the loss is as per the laboratory.

FECAL MICROSCOPIC CYTOLOGY

Frequent Indications • Large or small intestinal diarrhea.

Advantages • Availability and ease of performing the test.

Disadvantages • Variable specificity for a particular causative factor.

Analysis • Thin, air-dried, fresh fecal smears are stained with NMB or Wright's stain and examined using high-power and oil immersion. Rectal and colonic mucosal scraping obtained with a curette is also a means of examining mucosal cells.

Normal Values • A mixed population of rod and cocci bacteria, few bacterial spores or yeast, occasional epithelial cells and amorphous debris.

Artifacts • Old fecal sample (white blood cells [WBCs] do not remain identifiable in feces for long times, and the bacterial population

changes and bacterial spores may increase). Fecal debris may resemble degenerate WBCs.

Drug Therapy • Administration of barium and psyllium fiber may make interpretation difficult, and antibiotics change bacterial flora composition.

Causes of Abnormalities • Fecal WBCs (specifically neutrophils) are observed with bacterial (e.g., salmonellosis, campylobacteriosis) and inflammatory mucosal disease. Transmural colitides occasionally have increased fecal WBCs. Fecal WBCs are an indication to culture for specific bacterial pathogens or to biopsy colonic mucosa. Eosinophils may be visible with allergic or parasitic colitis. Increased numbers of yeast, fungal organisms, or a uniform population of bacteria may help identify the cause of diarrhea in a patient.

FECAL OCCULT BLOOD

Rare Indications • To detect GI bleeding that is not apparent grossly (i.e., melena, hematochezia).

Disadvantages • See Artifacts.

Analysis • Fresh feces are smeared on a test pad. The patient must have been on a meat-free diet for at least 3 days before the feces are obtained. Sensitivity varies markedly between assays.

Normal Values • See Artifacts.

Artifacts • Falsely decreased: sampling unmixed feces (blood may not be distributed homogeneously throughout the feces) and vitamin C supplementation. Falsely increased: diets containing fresh meats (i.e., hemoglobin) or fresh uncooked vegetables (i.e., peroxidases), which cause a positive reaction.

Causes of Fecal Occult Blood • Bleeding into the GI tract at any level and as the result of any cause may result in fecal occult blood. GI blood loss of volumes of 2 ml blood/30 kg body weight will give positive results.

FAT ABSORPTION TEST

Rare Indications • To detect and distinguish maldigestion from malabsorption in chronic small intestinal diarrhea or unexplained weight loss. Despite low cost and availability, this test has many false-negative and false-positive results and is *not recommended.*

Analysis • The test is described in prior editions.

BENTIROMIDE

Rare Indications • Because of the accuracy and the availability of the TLI test, the bentiromide (BT-PABA) test is rarely used and is *not recommended.*

TRYPSIN-LIKE IMMUNOREACTIVITY

Common Indications • Chronic small bowel diarrhea or weight loss.

Advantages • High sensitivity and specificity for EPI. Only need one serum sample that does not require special or cumbersome handling procedures, and most large veterinary diagnostic laboratories can perform this test for dogs.

Disadvantages • Currently the only laboratory offering the fTLI test (for cats) is the GI Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4474.

Analysis • Performed on serum using ELISA. See Appendix I for availability.

Normal Values • Dogs, 5 to 35 μg/L (Figure 9-4); cats, 12 to 82 μg/L.

Danger Values • None.

Artifacts • Theoretically, EPI caused by an obstructed pancreatic duct instead of acinar cell atrophy would yield a normal or even increased serum TLI value.

Drug Therapy That May Alter TLI • Drugs causing acute pancreatitis (see Table 9-3) might increase serum TLI. Oral pancreatic enzyme supplementation does not affect serum TLI concentrations.

Causes of Decreased TLI • A serum TLI concentration less than 2.5 μg/L (dog) or

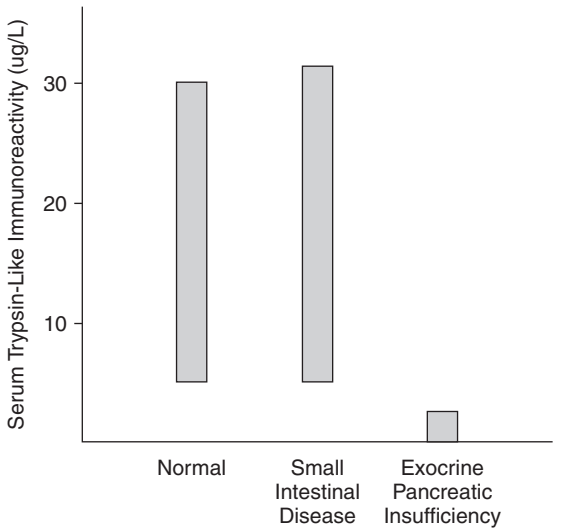

FIGURE 9-4. Typical ranges of trypsin-like immunoreactivity (TLI) values in normal dogs, dogs with small intestinal disease, and dogs with exocrine pancreatic insufficiency (EPI). (Modified from Williams DA: Sensitivity and specificity of radioimmunoassay of serum trypsin-like immunoreactivity for the diagnosis of canine exocrine pancreatic insufficiency, *J Am Vet Med Assoc* 192:195, 1988.)

8 µg/L (cat) is generally considered diagnostic for EPI, and TLI is considered to be the test of choice for EPI (see Figure 9-4). Subclinical canine EPI may be suspected by finding intermediate values (>2.5 µg/L and <5.0 µg/L). In such cases repeated testing should be performed. Some dogs will later develop EPI, whereas others will not.

Causes of Increased TLI • Values greater than 50 µg/L in dogs and greater than 100 µg/L in cats may occur with pancreatitis, renal failure, prerenal azotemia (may increase two times), and malnutrition. The fTLI test seems to be more specific for the diagnosis of pancreatitis in cats than amylase or lipase; however, the fTLI test is not clearly as sensitive or specific for feline pancreatitis as pancreatic biopsy. In dogs, TLI seems to increase early in pancreatitis but then returns to reference ranges.

ORAL GLUCOSE ABSORPTION TEST

Rare Indications • It is *not recommended* for evaluating the GI tract. See prior editions for description.

STARCH DIGESTION TEST

Rare Indications • Same as for oral glucose absorption test.

D-XYLOSE ABSORPTION TEST

Rare Indications • It is *not recommended* for evaluating the GI tract. It is described in prior editions.

SUGAR PERMEABILITY TESTING

Rare Indications • To confirm small intestinal disease that is not obviously apparent with other testing.

Advantages • This test is a sensitive means of detecting intestinal dysfunction that may not be obvious clinically. It may also detect post-treatment or post-dietary changes in intestinal function that are not obvious clinically.

Disadvantages • Currently one cannot correlate a specific disease with test results. The only laboratory currently offering this test for dogs is the GI Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4474.

Analysis • A solution of 2 to 5 sugars obtained from the laboratory is administered orally. Six hours later a spot urine sample is obtained and placed in a container with sodium azide to prevent bacterial degradation of the sugars. The concentration of the sugars is determined with high-pressure liquid chromatography, and the ratio of specific sugars is calculated. Results are interpreted as per the laboratory.

SERUM VITAMIN B_{12} AND SERUM FOLATE

Occasional Indications • Chronic small bowel diarrhea, unexplained weight loss, or uncertain but suspected small intestinal disease.

Advantages • Need only one serum sample.

Disadvantages • Uncertain sensitivity and specificity for small intestinal disease, ARE,

or EPI. This test should be used as an adjunct to other tests for maldigestion and malabsorption syndromes. The test is specific for cobalamin deficiency.

Analysis • Measured in serum by bioassay or immunoassay. "No boil" methods are unreliable in dogs. Serum should be transported in a covered tube.

Normal Values • Depend on the laboratory. Normal ranges vary widely between laboratories. The particular laboratory must validate the assay for dogs or cats.

Danger Values • None.

Artifacts • Falsely decreased vitamin B_{12}: degradation caused by exposure of serum to sunlight.

Drug Therapy That May Alter Serum Vitamin B_{12} Concentrations • Dietary content or vitamin supplementation of vitamin B_{12} and folate can affect serum concentrations. Drugs that affect intestinal bacterial concentrations (antibiotics or antacids) may also alter values.

Causes of Decreased Serum Vitamin B_{12} Concentrations • The major recognized reasons for decreased serum B_{12} concentrations in dogs and cats are ileal disease or resection (rare), EPI, intestinal mucosal disease, ARE, and in cats, hepatic disease. The major differentiation to be made is among EPI, mucosal disease, and ARE; therefore, decreased serum B_{12} is an indication for serum TLI. Not all dogs with EPI, mucosal disease, or ARE have decreased serum vitamin B_{12}. Cats with EPI, severe small intestinal disease (e.g., lymphoma, inflammatory bowel disease), and some hepatic diseases (e.g., idiopathic hepatic lipidosis) can have very low B_{12} concentrations. Finding a significantly decreased serum B_{12} concentration can be an indication of small intestinal disease in animals that were previously not suspected to have such disease.

Causes of Increased Serum Vitamin B_{12} Concentrations • Vitamin B_{12} supplementation.

Causes of Decreased Serum Folate • Severe mucosal disease of the proximal small intestine decreases serum folate. Not all patients with such disease have decreased folate levels.

Causes of Increased Serum Folate • ARE, EPI, and dietary supplementation are probably the major causes. Many patients with these diseases do not have increased folate levels. The combination of low vitamin B_{12} plus increased folate is an indication to treat for ARE.

HYDROGEN BREATH TEST

Rare Indications • Chronic small bowel diarrhea or unexplained weight loss. The test detects hydrogen production as a by-product of bacterial fermentation of carbohydrates. Increase in hydrogen production indicates ARE or carbohydrate malabsorption.

Advantages • Ease of the test and its lack of invasiveness.

Disadvantages • Need for special equipment, unknown sensitivity and specificity, multiple confounding factors. This is not a routine diagnostic test, because it requires special hydrogen analysis equipment.

Analysis • The concentration of hydrogen in expired air is measured after ingestion of food or a carbohydrate such as D-xylose or lactulose.

Normal Values • Dogs, varies with laboratory analysis and the carbohydrate administered.

Artifacts • Abnormal intestinal motility may delay or hasten expiration of hydrogen owing to colonic bacterial fermentation of carbohydrates.

Drug Therapy That May Alter Hydrogen Breath Test • Antibiotics and drugs that delay intestinal transit may cause decreased or delayed expiration of hydrogen.

Causes of Decreased Expired Hydrogen • Normal.

Causes of Increased Expired Hydrogen • Carbohydrate malabsorption and ARE may increase expired hydrogen. The only source of hydrogen is bacterial fermentation of carbohydrates. The sensitivity and specificity of this test for ARE in dogs are unknown.

FECAL SMEAR (WET MOUNT) FOR PARASITES

Common Indications • A screen for parasites and parasitic ova; any patient with diarrhea, melena, hematochezia, fecal mucus, weight loss, or vomiting.

Advantages • Availability, ease of performing the test, and low cost.

Disadvantages • Need for fresh feces and the frequency with which parasites and their ova or cysts are not detected.

Analysis • A thin smear is made of very fresh (< 5 minutes old) feces, usually mixed with a drop of saline solution and coverslipped to prevent dehydration. It should be examined immediately. If protozoa are visible and better cytologic detail is desired, a drop of Lugol's iodine or Dobell and O'Connor's iodine may be placed at the corner of the coverslip.

NOTE: Iodine kills protozoa, thus stopping motility.

Normal Values • Dogs and cats, no parasites or ova.

Artifacts • Cooling of the slide or dehydration inhibits the motility of some protozoa and bacteria.

Drug Therapy That May Alter Results • Orally administered compounds containing kaolin, pectin, barium sulfate, bismuth, and other intestinally active compounds (e.g., cathartics, enemas) may make it difficult to find and identify parasites, ova, and cysts.

Parasites, Bacteria, and Ova That May Be Identified • This test is most useful in identifying *Giardia* spp., *Tritrichomonas* spp., *Entamoeba histolytica, Balantidium coli, Strongyloides stercoralis*, and *Aleurostrongylus abstrusus*. Any ova may be found, but this test may be useful for detecting *Spirocerca lupi* and *Trichuris vulpis* ova. With oil immersion, small motile bacterial spirochetes in conjunction with fecal WBCs suggest *Campylobacter* spp. as a possible cause.

FECAL FLOTATION

Common Indications • As for fecal smear.

Advantages • Sensitivity, availability, and low cost.

Analysis • Feces are well mixed with either a saturated sugar solution or a zinc sulfate solution (Leib, 1999). (Zinc sulfate solution is made by mixing 331 g $ZnSO_4$ • 7 H_2O in 1 L water to attain a specific gravity of 1.18 to 1.20 [as determined with a hydrometer]. This is supposedly the best fecal flotation technique for *Giardia* spp. because it does not distort the cysts.) Ova and cysts are allowed to rise to the surface and are retrieved with a coverslip. Samples for *Giardia* detection should be examined within 15 minutes to avoid distortion and lysis of cysts. Centrifugation of the sample increases the sensitivity of the procedure. Samples that will be sent to an outside laboratory for analysis may be refrigerated (not frozen) for 1 to 2 days or preserved by mixing 1 part feces with 3 parts sodium acetate—acetic acid—formalin. This is prepared by mixing 1.5 g sodium acetate + 2 ml glacial acetic acid + 4 ml 40% formaldehyde solution + 92.5 ml water (Kirkpatrick, 1987).

Normal Values • Dogs and cats, no ova or oocysts present.

Artifacts • Falsely decreased: Diarrhea may decrease ova concentration within a sample.

Parasite Ova and Cysts That May Be Identified • *Ancylostoma* spp., *Toxocara* spp., *Toxascaris leonina*, *T. vulpis, S. lupi, Physaloptera rara* (using dichromate solution), *Capillaria aerophilia*, *Capillaria plica, Onciolo canis, Dioctophyme renale, Isospora* spp., *Giardia* spp., *Toxoplasma gondii, Cryptosporidium* spp., *Paragonimus kellicotti,* and some tapeworms.

FECAL SEDIMENTATION

Rare Indications • Same as for fecal smear and flotation, especially if flukes are being considered. If feces contain excessive fat, formalin and ethyl acetate is probably better than water sedimentation.

Disadvantages • Requires more time than direct fecal smear or fecal flotation.

Analysis • Feces are mixed with the sedimentation solution (e.g., water), usually strained once or twice to remove large debris, and

allowed to settle for 30 minutes to 2 hours. The sediment is then examined microscopically. When formalin and ethyl acetate is used, the strained feces are centrifuged, the pellet is resuspended in 9 ml of 5% formalin solution, 3 ml ethyl acetate is added, and the mixture is shaken vigorously. This is recentrifuged, the debris at the formalin and ethyl acetate interface is discarded, and the sediment is then examined (Kirkpatrick, 1987).

Normal Values • Dogs and cats, no ova.

Artifacts • Same as under Fecal Flotation.

Parasite Ova That May Be Identified • All the ova that may be found by fecal flotation, plus *Alaria canis* and *Nanophyetus salmincola*.

FECAL *GIARDIA* DETECTION

Occasional Indications • Chronic diarrhea, unexplained weight loss, intermittent bilious vomiting, or when *Giardia* is suspected clinically and multiple (i.e., at least 3) zinc sulfate flotations using centrifugation are negative. Techniques include duodenal aspiration and cytology, fecal ELISA antigen test (e.g., ProSpecT Microplate ELISA Assay for *Giardia*, Alexon, Lenexa, KS), and IFA (e.g., MeriFlour *Cryptosporidium/Giardia*, Meridian Diagnostics, Cincinnati, OH) performed on feces.

Advantages • Provides additional methods for detection of *Giardia*.

Disadvantages • Duodenal aspirates require surgery or endoscopy.

Analysis • Duodenal fluid aspirates require fresh direct wet-mount observation of motile trophozoites. Fresh samples should be used for analysis with fecal ELISA and fecal IFA.

Normal Values • No trophozoites or fecal antigen present.

FECAL *CRYPTOSPORIDIUM* DETECTION

Rare Indications • Chronic diarrhea. Cats (especially with feline immunodeficiency virus [FIV] infection) may be more likely to have cryptosporidiosis than dogs, but the prevalence of this disorder is currently unknown.

Disadvantages • Oocysts are small and may be difficult to find.

Analysis • Fresh fecal samples should be sent to a referral laboratory experienced in finding *Cryptosporidium*. Special fecal flotation techniques, direct fecal smears stained with an acid-fast stain, or ELISA methodology (e.g., ProSpecT *Cryptosporidium* Microplate Assay, Alexon, Lenexa, KS) can be used. The ELISA methodology appears to be the most sensitive.

Normal Values • Dog and cat feces should be negative for *Cryptosporidium*.

HEPATIC ABNORMALITIES

Hepatic disease may be heralded by relatively specific signs (e.g., hepatomegaly, microhepatia, icterus, ascites, hepatic encephalopathy associated with meals) or may be associated with nonspecific signs (e.g., depression, weight loss, anorexia, vomiting). The latter are common presenting complaints of many diseases, which is why serum biochemistry profiling is indicated in patients with chronic signs or evidence of systemic disease. It is important to note that no consistent signs or laboratory abnormalities are found in all patients with hepatic disease. When screening for hepatic disease, one should request at least CBC, serum ALT, SAP, gamma-glutamyl transpeptidase (GGT), total bilirubin, albumin, cholesterol, BUN, glucose, urinalysis, and abdominal imaging. Hepatic function tests (bile acids, ammonia tolerance, clotting times, and others) ultrasonographic examination, hepatic biopsy, or contrast angiography and portography is usually necessary for definitive diagnosis. Abnormalities in hepatic-specific enzymes may result from primary hepatic disease but also occur because of secondary hepatic involvement from a primary nonhepatic disease (e.g., glucocorticoid hepatopathy, inflammatory bowel disease, pancreatitis). After identifying abnormalities in ALT, aspartate transferase (AST), SAP, or GGT, one should investigate first for a primary nonhepatic disease, because nonhepatic disease is the most common cause of increased values. In such cases the liver usually has reactive but reversible degenerative changes. Laboratory tests should be used for two main purposes: (1) to identify the presence of hepatic disease and (2) to determine if a

biopsy or radiographic contrast procedure is indicated.

Microhepatia: Small Liver

A small liver suggests atrophy (due to portosystemic shunts, hepatic arteriovenous [AV] fistulas), fibrosis and cirrhosis, or diffuse massive hepatic necrosis (Figure 9-5). Radiographically, hepatic atrophy tends to be characterized by sharp borders as opposed to the rounded or blunted hepatic margins typically associated with fibrosis and cirrhosis. Some patients with primary hepatic fibrosis severe enough to cause portal hypertension also have sharp hepatic margins, however. Many patients with marked hepatic atrophy due to portosystemic shunting are relatively young (<1 to 2 years) and have had signs of hepatic disease since (or before) weaning, whereas most patients with cirrhosis are middle-aged or older and clearly have late onset of clinical signs. Hepatic AV fistula is an uncommon cause of microhepatia, but it is usually diagnosed in dogs less than 2 years of age. However, some dogs are first diagnosed as having a single congenital portosystemic shunt when they are more than 10 years old. Likewise, although acquired portosystemic shunts are classically thought of as occurring in older dogs, they can be diagnosed in dogs less than 6 months old.

Hepatic atrophy causes abnormalities in hepatic function tests (e.g., bile acids, ammonia, or ammonium chloride tolerance test) but may yield normal or abnormal ALT, SAP, BUN, and serum albumin. A single normal or abnormal hepatic function test result does not mean that other hepatic function tests will have similar results. Preprandial and postprandial serum bile acid concentrations are sensitive function tests. (NOTE: Cholestatic diseases also increase bile acids; therefore, bile acids are not a "pure" test of hepatic function.) However, if hepatic disease is strongly suspected and the serum bile acid concentrations are not as high as anticipated, one should not hesitate to perform other tests to characterize the liver. If hepatic atrophy is likely, abdominal ultrasonography, contrast portography, hepatic biopsy, or a combination of these might be considered.

Small livers with clearly rounded or blunt hepatic margins are usually cirrhotic. Significant increases in serum ALT and SAP are often present, but some dogs with marked hepatic cirrhosis have normal hepatic enzymes. Serum albumin and BUN are more variable. If cirrhosis appears likely, a biopsy is indicated. Most cirrhotic livers can be identified grossly by their nodular or "cobblestone" appearance. However, significant fibrosis can be present without major gross changes, and severe nodular hyperplasia may resemble a cirrhotic liver. Acquired multiple shunts visible at laparoscopy or laparotomy are usually due to cirrhosis but can be secondary to congenital hepatic AV fistula, venoocclusive disease, or portal vein obstruction. If the liver is not clearly cirrhotic or fibrotic, a mesenteric venoportogram may be indicated in patients with acquired shunting.

Hepatomegaly: Enlarged Liver

Focal or asymmetric hepatic enlargement generally necessitates further laboratory investigation, imaging, and possibly biopsy. Neoplasia is a prominent but not invariable cause of focal hepatomegaly. The magnitude of the enlargement is not prognostic.

Generalized hepatomegaly necessitates careful clinicopathologic evaluation. Hepatomegaly may be the result of primary or secondary hepatic disease. Diagnosis may be confirmed with a history of exposure to certain toxins (Tables 9-6 and 9-7) or diagnosis of a systemic disease (e.g., hyperadrenocorticism) known to affect the liver. *Changes in ALT, SAP, hepatic function tests, and hepatic size, although suggestive of hepatic disease, are not diagnostic of specific entities*. This is true even in breeds with specific predispositions (e.g., Doberman pinschers, Bedlington terriers). Changes in the SAP or serum ALT may also be the result of primary nonhepatic disease (e.g., hyperadrenocorticism, inflammatory bowel disease, diabetes mellitus, heart failure). A definitive diagnosis usually requires hepatic biopsy. The clinician should first seek to rule out nonhepatic causes of secondary hepatic dysfunction. Hepatic biopsy should be considered in patients with obviously significant hepatic disease, those that do not have hyperadrenocorticism, and those that have persistent (more than 1 month) changes in serum ALT or SAP consistent with chronic or progressive hepatic disease or abnormal hepatic function tests (see Figure 9-5). It is not always possible to make these distinctions accurately; therefore, whenever hepatic biopsy is performed via laparotomy or laparoscopy, the rest of the abdomen should be explored and other organs sampled if the clinician

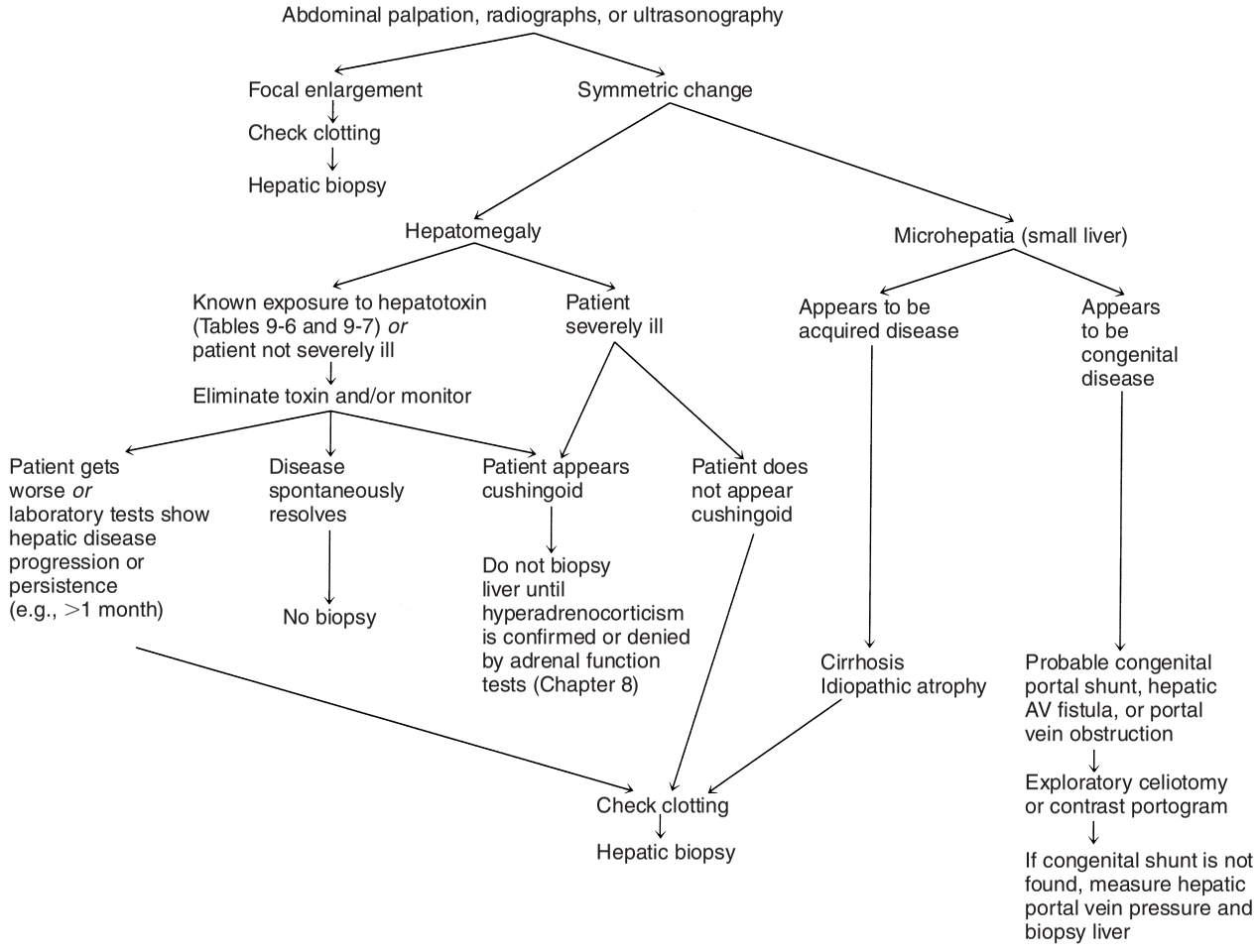

FIGURE 9-5. Diagnostic approach to altered hepatic shape or size in dogs and cats.

TABLE 9-6. Drugs That Have Been Documented or Suspected to Cause Increased Alanine Aminotransferase (ALT) Levels Due to Hepatic Disease

Acetaminophen *(important)* especially cats
Amiodarone
L-Asparaginase
Azathioprine
Barbiturates *(important)*
Carprofen
Doxycycline
Diazepam
Erythromycin estolate
Glucocorticoids *(dogs only) (important)*
Griseofulvin
Halothane
Ibuprofen
Itraconazole
Ketoconazole
Mebendazole
6-Mercaptopurine
Methimazole
Methotrexate
Methoxyflurane
Nitrofurantoin
Oxacillin
Oxibendazole
Phenobarbital *(important)*
Phenylbutazone
Phenytoin
Primidone *(important)*
Quinidine
Salicylate
Salicylazosulfapyridine
Sulfonamides
Tetracycline
Thiacetarsemide *(important)*
Trimethoprim-sulfa drug *(important)*

NOTE: These drugs do not reliably cause hepatic disease. In a patient with an increased ALT that is receiving one of these drugs, the medication probably should be stopped, if possible, and the ALT rechecked 2 to 4 weeks later. Those drugs that most reliably increase ALT are marked *(important)*. The other drugs are less consistent but may still cause severe hepatic disease. Almost any drug could cause an increased ALT in a particular patient.

TABLE 9-7. Drugs That Have Been Documented or Suspected to Cause Cholestasis or Hepatic Enzyme Induction Resulting in Increased Serum Alkaline Phosphatase (SAP) Levels

Anabolic steroids/androgens
Asparaginase
Azathioprine
Barbiturates *(important)*
Cephalosporins
Cyclophosphamide
Dapsone
Erythromycin estolate
Estrogens
Glucocorticoids *(important in dogs only)*
Gold salts
Griseofulvin
Halothane
Ibuprofen
6-Mercaptopurine
Methimazole
Methotrexate
Nitrofurantoin
Oxacillin
Oxymetholone
Phenobarbital *(important)*
Phenothiazines
Phenylbutazone
Phenytoin
Primidone *(important)*
Progesterone
Salicylates
Sulfur
Testosterone
Tetracyclines
Thiabendazole
Trimethoprim-sulfa drug
Vitamin A

NOTE: Those drugs that most reliably increase SAP are marked *(important)*. The other drugs are less consistent.

doubts their involvement. Fine-needle aspirates with cytology are sometimes useful in detecting diffuse hepatic infiltrative disease and hepatic lipidosis (see Color Plate 5E); however, fine-needle aspirates (even when guided by ultrasound) often miss infiltrative processes, and a negative cytologic finding never excludes an infiltrative disease in the liver. In general, laparoscopy allows for much superior hepatic biopsies compared with ultrasound-guided biopsies.

Hepatic Encephalopathy

Abnormal behavior, sometimes associated with eating, may be caused by hepatic encephalopathy, although hypoglycemia, primary CNS disease, and epilepsy must also be considered. Whenever possible, glucose should be measured on blood obtained during an episode. Evaluation of hepatic function is indicated in patients with behavioral changes, transient blindness, seizures, coma, or vague CNS abnormalities. Congenital (e.g., portosystemic shunt) and severe acquired hepatic disease (e.g., cirrhosis) may cause encephalopathy. Routine biochemical profiling may be suggestive, but hepatic function testing is mandatory, because these diseases may not significantly change serum ALT, SAP, albumin, BUN, glucose, or bilirubin determinations. Resting plasma ammonia concentrations are meaningful only if they are increased. A patient in an episode of hepatic encephalopathy may have increased or normal resting plasma ammonia concentrations. Ammonia tolerance testing (ATT) and pre- and postprandial serum bile acid concentrations appear to be the most sensitive

and specific tests for hepatic dysfunction that causes hepatic encephalopathy. A very rare congenital urea cycle enzyme deficiency may cause hepatic encephalopathy and hyperammonemia without affecting enzymes or bile acids. Analysis of urea cycle enzymes in biopsy samples is necessary for diagnosis.

Icterus

Icterus is detected at physical examination or when serum or plasma is inspected at the laboratory. Hyperbilirubinemia always denotes hepatobiliary or hematopoietic disease (Figure 9-6). Hepatic and hematopoietic diseases are not always associated with icterus, and disease in either system may be secondary to other disorders. The presence or absence of icterus is not diagnostic or prognostic. Sepsis, pancreatitis, and inflammatory bowel disease sometimes cause secondary hepatic dysfunction that may include icterus.

TOTAL SERUM BILIRUBIN

Occasional Indications • Icterus (on either physical examination or inspection of nonhemolyzed serum or plasma), bilirubinuria (any amount in a cat or significant amounts in a dog), or suspected hepatic disease that is not apparent on other tests. The sclera have detectable icterus when the serum bilirubin is greater than 3 to 4 mg/dl, and the plasma is icteric when the serum bilirubin is greater than 1.5 to 2 mg/dl.

> NOTE: Icterus is absent in many animals (especially dogs) with hepatic disease. Serum bilirubin is not a sensitive test for hepatic disease.

Measurement of direct (conjugated) and indirect (unconjugated) bilirubin fractions is not useful and should not be done. Hemolytic, hepatic, and biliary tract diseases have unpredictable variation in the amount of each fraction. Other tests are required to identify the cause of hyperbilirubinemia.

Analysis • Measured in serum or heparinized plasma by spectrophotometric and dry reagent methods. The latter require dilutions if the bilirubin is greater than 7.5 mg/dl. Bilirubin is stable at 4° C for 7 days if not exposed to bright light. Measurement of urine bilirubin is discussed in Chapter 7.

Normal Values • Dogs, less than 1.0 mg/dl; cats, less than 1.0 mg/dl.

Danger Values • Dogs, uncertain, but values greater than 20 mg/dl cause concern (i.e., kernicterus); cats, unknown.

Artifacts • Exposure to bright sunlight or fluorescent lighting can decrease bilirubin by 50% per hour. See Introduction to Serum Chemistries.

Drug Therapy That May Alter Serum Bilirubin • Decreased bilirubin may be caused by drugs that cause hepatic enzyme induction (e.g., phenobarbital). Increased bilirubin may be the result of drugs causing hemolytic anemia (Table 9-8) or acute hepatic necrosis (see Table 9-6).

Causes of Hypobilirubinemia • Do not exist.

Causes of Hyperbilirubinemia • Hemolytic disease and hepatobiliary disease are the two main causes (see Figure 9-6). A CBC should be determined in every icteric patient to help rule out hemolytic disease. RBC numbers must decrease rapidly and significantly to cause clinical icterus. Very regenerative anemias may suggest that icterus is due to immune-mediated hemolytic anemia (IMHA). Reticulocytosis, hemoglobinemia, hemoglobinuria, erythrocytic autoagglutination, spherocytosis, positive Coombs' test results, splenomegaly, or hepatomegaly are often present. See Chapter 3 for further discussion of IHA and other regenerative anemias (e.g., Heinz body, zinc intoxication, *Haemobartonella*). Bilirubinuria theoretically should be absent in hemolytic disease but is often present in IHA because canine kidneys conjugate bilirubin. The clinician must not be misled by increases in ALT because severe, acute hemolytic anemia may cause increased ALT (ostensibly caused by acute hepatic hypoxia).

Severe hepatic disease (especially acute necrosis) is sometimes accompanied by DIC and subsequent hemolytic anemia. These cases may be difficult to distinguish from IHA. However, anemia caused by DIC is usually not as regenerative as in IHA; in addition, the presence of RBC fragments, thrombocytopenia, increased fibrin degradation products (FDP), decreased antithrombin III, prolonged clotting time, and abnormal hepatic function

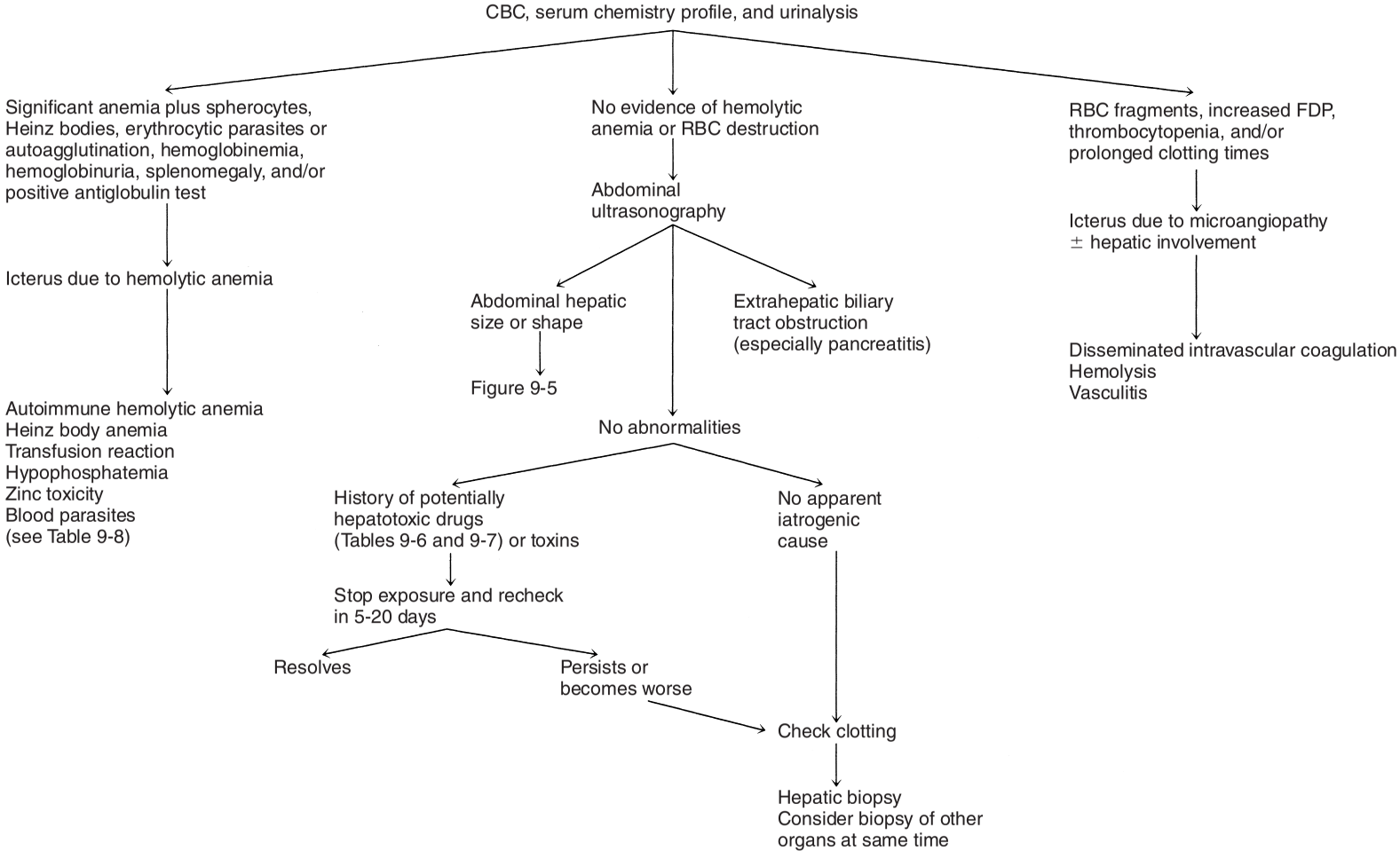

FIGURE 9-6. Diagnostic approach to hyperbilirubinemia in dogs and cats. *CBC,* Complete blood count; *RBC,* red blood cell; *FDP,* fibrin degradation products.

TABLE 9-8. Selected Substances That Have Been Documented or Suspected to Cause Hemolytic Anemia

Acetaminophen *(especially cats)*
Benzocaine *(especially cats)*
Cephalosporins
Dapsone
Methylene blue *(especially cats)*
Nitrofurantoin
Onions
Penicillins
Phenacetin *(especially cats)*
Phenazopyridine
Phenylbutazone
Sulfonamides
Vaccinations
Vitamin K_3 *(cats)*
Zinc

NOTE: These substances do not always cause anemia, but they have the potential and should be withdrawn, if possible, in patients with hemolytic anemia.

tests usually allows differentiation, as do vomiting, abdominal pain, and encephalopathy when present.

Dogs and cats often have relatively severe hepatic disease before icterus is observed; however, the magnitude of the total serum bilirubin is not prognostic or diagnostic. Secondary hepatic disease (reactive disease or so-called *bystander phenomenon* as the result of septicemia, toxemia, or inflammation) may have icterus similar to that occurring in primary hepatic disease. Certain bacterial endotoxins and acute-phase inflammatory mediators are thought to alter normal bilirubin metabolism and cause increases in total bilirubin concentrations.

Most feline hepatic diseases cause icterus; the most common conditions include hepatic lipidosis, cholangitis and cholangiohepatitis, hepatic lymphoma, and FIP. Icterus in cats is an indication for CBC and a serum biochemistry panel. Icterus in cats that is not caused by hemolysis usually indicates a hepatic biopsy, because most of these cats have primary hepatic disease. Biopsy is necessary to differentiate causes and institute specific treatment.

Common causes of nonhemolytic icterus in dogs include pancreatitis obstructing the bile duct, cholecystitis, chronic hepatitis, hepatic lymphoma, acute hepatic necrosis, hepatic cirrhosis, and intrahepatic cholestasis. Icterus in dogs is an indication for CBC and a serum biochemistry panel (to include at least ALT, SAP, BUN, cholesterol, and albumin). Imaging (radiographs, ultrasonography, or both) is indicated to help determine if primary hepatic disease or biliary tract obstruction exists. If primary hepatic disease is diagnosed, hepatic biopsy is usually indicated. If pancreatitis is present, surgery is not indicated unless a chronic bile duct obstruction necessitates bypassing the common bile duct with a cholecystoduodenostomy (rarely needed) or a pancreatic abscess is present. If coexisting extrahepatic disease is found, it should be investigated.

ALANINE TRANSFERASE

ALT was formerly known as *serum glutamic-pyruvic transaminase* (SGPT).

Common Indications • Systemic disease including weight loss, hepatomegaly, vomiting, diarrhea, icterus, ascites, depression, and anorexia; also, as a screening procedure for hepatic disease in any patient with undiagnosed illness. Most patients with known chronic hepatitis should undergo periodic ALT determinations to monitor the problem.

Advantages • Specificity for the liver.

Disadvantages • Lack of sensitivity (i.e., patients with significant hepatic disease such as cirrhosis or hepatic neoplasia may have normal ALT) and inability to distinguish among different hepatic diseases or when there is secondary nonhepatic disease involvement.

Analysis • Measured in serum (heparinized plasma in selected assays) by spectrophotometric and dry reagent methods. ALT is stable in separated serum for approximately 1 (at 22° C) to 7 (at 4° C) days.

Normal Values • Serum enzyme activity may vary markedly among laboratories, depending on the technique and the units used.

Danger Values • Despite correlation between ALT and active hepatic damage, no correlation exists between ALT and hepatic function; hence, no danger values exist.

Artifacts • See Introduction to Serum Chemistries.

Drug Therapy That May Alter Serum ALT • Any drug causing hepatocellular damage (i.e., drug-induced hepatopathy) may cause increased ALT. The list of all drugs suspected to cause increased ALT is extensive

and includes many that are safe in the majority of patients. A list of selected drugs documented to cause increased ALT in human beings, dogs, and cats is given in Table 9-6. Administration of one of these drugs does not automatically explain an increased ALT, however.

NOTE: A patient can have an idiosyncratic reaction to almost any drug, causing an increased ALT.

Causes of Decreased ALT • Not significant.

Causes of Increased ALT • Increase in ALT is principally caused by hepatocellular damage from any cause (Table 9-9). RBCs and striated muscle cells contain small amounts of ALT, and damage to these may cause relatively minor increases (i.e., less than two to three times normal) in serum ALT, as may exercise. Dogs with muscular dystrophy may have major increases in ALT, but should also have increases in AST and creatine kinase (CK) values.

Hepatocytes contain substantial amounts of ALT in the cytosol, and major increases in serum ALT (i.e., three or more times normal) indicate hepatocellular leakage of the enzyme but do not always signify primary or irreversible hepatic disease. Hepatic disease may have normal to significantly increased serum ALT activity. The magnitude of the increase in ALT does not correlate with the seriousness of the hepatic disease and is not a prognostic indicator unless a specific disease is being considered. The serum ALT half-life is approximately 1 to 2 days or less, and serum ALT is expected to decrease over 1 to 2 weeks once active hepatic damage ceases. It is thought that ALT remains elevated during hepatic regeneration.

After increased serum ALT is identified, many factors must be considered (Figure 9-7). If no other evidence of disease is found, the increased ALT indicates the need for periodic monitoring because it may be the first detectable sign of significant hepatic disease. If other abnormalities consistent with hepatic disease are found, the approach is like that in any other patient with hepatic disease. Common causes of serum ALT more than three times normal include hepatic anoxia, poor hepatic perfusion, spontaneous and surgical trauma (e.g., hit by a car, surgery), chronic hepatitis, cirrhosis, cholangitis and cholangiohepatitis, acute biliary obstruction, hepatic necrosis as the result of any cause, acute pancreatitis, hepatic neoplasia, sepsis, and certain drugs. Sepsis, especially septicemia and toxemia, may secondarily damage hepatocytes. Abdominal inflammation may do the same. The pancreas is close to the liver, and inflammation in the pancreas may cause mechanical damage to the liver. In Doberman pinschers, Bedlington terriers, Dalmatians, and West Highland white terriers, a persistently increased serum ALT suggests chronic hepatitis that may or may not be associated with increased hepatic copper concentrations.

ASPARTATE TRANSFERASE

AST was formerly known as *serum glutamic-oxaloacetic transaminase* (SGOT).

TABLE 9-9. Selected Causes of Increased Serum Alanine Aminotransferase Levels

DOGS	CATS
Hepatic Parenchymal Disease	**Hepatic Parenchymal Disease**
Cholangitis	Cholangitis
Cholangiohepatitis	Cholangiohepatitis
Cirrhosis	Feline infectious peritonitis (FIP)
Copper storage disease	Hepatic lymphoma
Hepatic malignancy	Cirrhosis
Chronic hepatitis	Hepatic toxin
Hepatic toxin	Trauma
Trauma	Pancreatitis
Pancreatitis	Hyperthyroidism
Other Disorders	**Other Disorders**
Anoxia because of anemia/shock	Anoxia because of anemia/shock
Iatrogenic (see Table 9-6)	Iatrogenic (see Table 9-6)

NOTE: Almost any disease affecting the liver can cause increased ALT levels. The disorders listed are those that may be more likely to cause a significant increase. However, any of these diseases can exist with minor or no increase in ALT values.

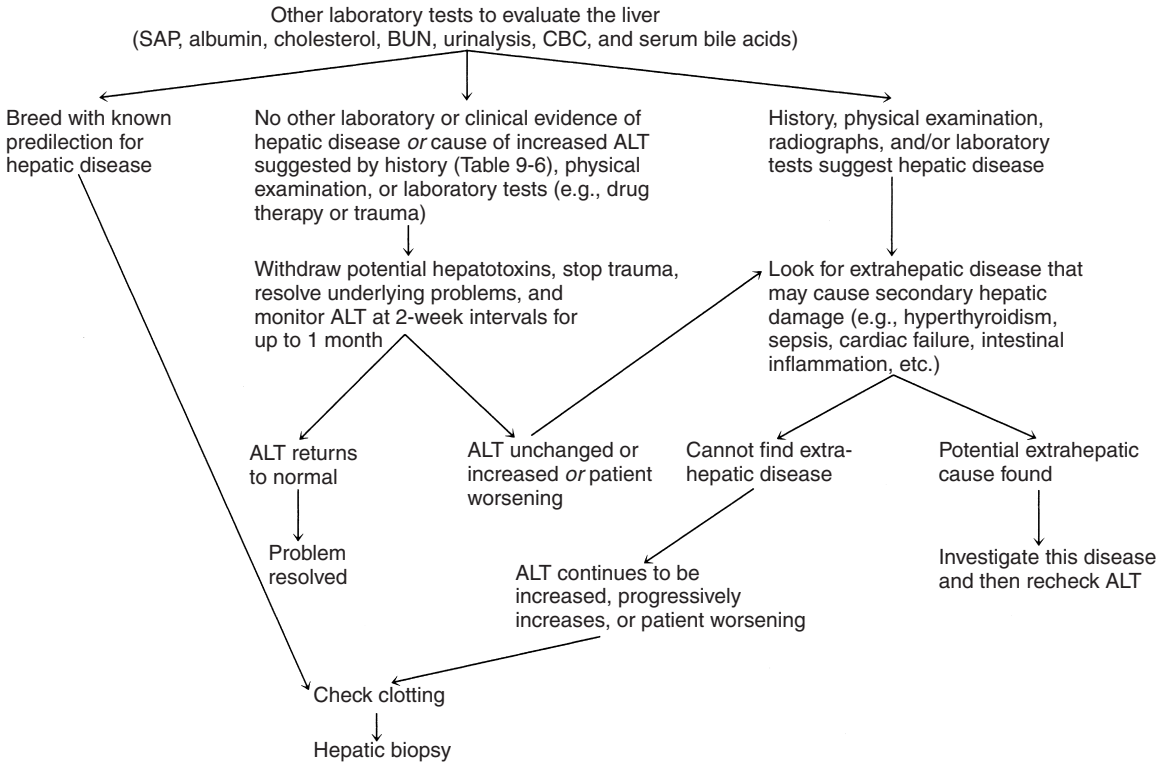

FIGURE 9-7. Diagnostic approach to increased alanine aminotransferase (ALT) in dogs and cats. *BUN,* Blood urea nitrogen; *CBC,* complete blood count; *SAP,* serum alkaline phosphatase.

Occasional Indications • Same as for ALT.

Disadvantages • Not as specific for the liver as ALT.

Analysis • Same as for ALT.

Drug Therapy That May Alter AST • Decreased AST may be caused by metronidazole therapy. Hepatotoxic drugs may cause increased AST (see Table 9-6).

Causes of Decreased AST • None.

Causes of Increased AST • Like ALT, AST is present in significant quantities in hepatocytes. Although ALT is present in the cytosol, AST is present in the mitochondria. Increased serum ALT reflects cell membrane damage and leakage; significant AST increases tend to reflect more serious hepatic damage because the mitochondria are not damaged as readily as is the cell membrane. AST is, however, present in significant quantities in many other tissues, including muscle and RBCs; therefore, increased AST is not as specific for hepatic injury as is increased ALT. Exercise and intramuscular (IM) injections may increase serum AST. The most common causes of increased AST include hepatic disease, muscle disease (inflammation or necrosis), or hemolysis (spontaneous or artifactual). Increased AST is an indication to check for ongoing hemolysis by measuring the hematocrit and observing the color of the plasma and serum on a centrifuged blood sample. If no hemolysis is found, the next step is to measure serum ALT to determine whether the increased AST is from the liver (significant increases in both ALT and AST suggest that AST increases are of hepatic origin).

SERUM ALKALINE PHOSPHATASE

Common Indications • Systemic disease, including weight loss, hepatomegaly, vomiting, diarrhea, ascites, icterus, depression, or

anorexia; also as a screen for hepatic disease and hyperadrenocorticism.

Advantages • Useful in evaluating the liver for subtle cholestatic disease.

Disadvantages • Affected by corticosteroids, bone lesions, and osteoblastic activity in young growing dogs.

Analysis • Measured in serum or heparinized plasma by spectrophotometric methods. Stability in heat (55° C) has been used to attempt to differentiate SAP of bone origin (i.e., heat sensitive) from SAP of hepatic origin (i.e., heat stable). It is difficult to obtain reproducible results with the heat-inactivation test; however, L-phenylalanine inhibits steroid-induced SAP and can be used to help determine if increased SAP is due to corticosteroids. Alternatively, cellulose acetate electrophoresis can separate the isoenzymes more definitively. The diagnostic usefulness of determining the percentage of steroid fraction is questionable, because dogs with various types of hepatic disease often have considerable steroid involvement.

Normal Values • May vary markedly from laboratory to laboratory. Immature dogs characteristically have SAP (bone origin) activities up to twice that of sexually mature dogs.

Danger Values • Because of the lack of correlation with hepatic function, no danger values exist for SAP.

Artifacts • See Introduction to Serum Chemistries.

Drug Therapy That May Increase SAP • Any drug that causes hepatic enzyme induction or cholestasis (see Table 9-7) may increase SAP. Glucocorticoids, primidone, and barbiturates typically increase SAP in dogs, but other drugs are less consistent. Although glucocorticoids can cause marked SAP increases in dogs, cats are almost never affected.

Causes of Decreased SAP • Not significant.

Causes of Increased SAP • SAP of bone origin is commonly increased (SAP less than three times normal) in dogs less than 6 to 8 months old. Bone disease (e.g., osteosarcoma, osteomyelitis) may increase SAP (usually a minor increase) and generally denotes a guarded prognosis from presumed metastatic disease in the bones.

Increased SAP is interpreted differently in dogs and cats (Table 9-10). Cats have less hepatocellular SAP, which is readily excreted by their kidneys. Therefore, any increase in feline SAP is considered significant, indicating further tests. Not all cats with hepatic disease have increased SAP. The major causes of increased SAP in cats are hepatic lipidosis, cholangitis and cholangiohepatitis, hyperthyroidism, and diabetes mellitus. SAP increases are generally more specific than GGT in cats with hepatic lipidosis (cats with lipidosis classically have very high SAP with little to no increase in GGT; however, this finding is not consistent enough to make a diagnosis). Hyperadrenocorticism (spontaneous and iatrogenic) very, very rarely increases SAP in cats. Increased SAP in a cat is an indication for serum thyroid hormone determination, urinalysis, blood glucose and serum ALT measurement, and perhaps a hepatic function test (e.g., bile acid). If hepatic disease is the apparent cause of the increased SAP, one must determine if hepatic biopsy is indicated (see the later discussion under Bile Acids).

The major causes of SAP values more than three times normal in dogs are hepatobiliary disease, hyperadrenocorticism, and therapy with glucocorticoids or anticonvulsants. Hepatic disease with increased SAP usually has a cholestatic component; however, this does not imply icterus or gross obstruction of the biliary tract. Intrahepatic cholestasis caused by diffuse or focal compression of bile canaliculi may occur in various hepatopathies, even those secondary to septicemia, toxemia, and chronic stress-induced vacuolar (i.e., hydropic change) hepatopathy. Acute hepatocellular necrosis can transiently increase SAP (usually less than five times normal). Extrahepatic biliary tract obstruction and enzyme induction caused by endogenous or exogenous glucocorticoids or drug administration may increase SAP more than 10 times normal. As with ALT, the magnitude of the increase in SAP does not correlate with the seriousness or prognosis of the disease.

In dogs it is important first to rule out young age, drug therapy, and hyperadrenocorticism to avoid performing an unnecessary hepatic biopsy (Figure 9-8). Hyperadrenocorticism can easily be confused with primary hepatic disease because it typically causes hepatomegaly, pu-pd, increased ALT, and sometimes increased serum bile acids. Unless a patient

TABLE 9-10. Causes of Increased Serum Alkaline Phosphatase Levels

DOGS	CATS
Biliary Tract Abnormalities	**Biliary Tract Abnormalities**
Pancreatitis	Same as for dogs
Bile duct neoplasia	
Cholelithiasis	
Cholecystitis	
Ruptured gallbladder	
Hepatic Parenchymal Disease	**Hepatic Parenchymal Disease**
Cholangiohepatitis	Cholangiohepatitis
Chronic hepatitis	Hepatic lipidosis
Copper storage disease	Hepatic lymphoma
Cirrhosis/fibrosis	Feline infectious peritonitis (FIP)
Hepatic neoplasia	
Lymphoma	
Hemangiosarcoma	
Hepatocellular carcinoma	
Metastatic carcinoma	
Toxic hepatitis	
Aflatoxin	
Other Disorders	**Other Disorders**
Diabetes mellitus	Diabetes mellitus
Hyperadrenocorticism	Hyperthyroidism
Chronic passive congestion because of right heart failure	
Diaphragmatic hernia	
Septicemia	
Ehrlichiosis*	
Young dog with bone growth Osteomyelitis*	
Iatrogenic (see Table 9-7)	Iatrogenic (see Table 9-7)*

NOTE: Almost any disease affecting the liver can cause increased SAP levels. The disorders listed are those that may be more likely to cause a significant increase. However, any of these can exist with minor or no increase in SAP values.
*Rarely of importance.

has signs of hepatic failure (i.e., icterus, hepatic encephalopathy, hypoglycemia, weight loss, vomiting, hypoalbuminemia, ascites, microhepatia), hyperadrenocorticism must be precluded by adrenal gland function testing. If a hepatic biopsy specimen is obtained from a patient with hyperadrenocorticism, vacuolar hepatopathy is documented.

GAMMA-GLUTAMYL TRANSPEPTIDASE

Occasional Indications • Same as for SAP. SAP appears to be more sensitive for hepatobiliary disease in dogs; however, in cats, GGT has slightly greater sensitivity and perhaps greater specificity for hepatic disease except hepatic lipidosis. Therefore, it is more frequently indicated in cats than in dogs. GGT is less influenced than SAP by secondary hepatic disease conditions or enzyme-inducing drugs. The use of SAP and GGT together has a higher predictive value of hepatic disease.

Analysis • Measured in serum, urine, and body fluids by spectrophotometric methods. GGT is stable in serum at 4° C for at least 3 days and at 20° C for up to 1 year.

Normal Values and Danger Values • Same as for SAP.

Artifacts • See Introduction to Serum Chemistries.

Drug Therapy That May Affect GGT • Same as for SAP.

Causes of Decreased GGT • Not significant.

Causes of Increased GGT • Causes are similar to increased SAP and tend to parallel the magnitude of the rise in SAP, but bone lesions are not recognized to increase GGT. It is induced by glucocorticoid therapy and certain drugs, as is SAP. In cats, GGT may increase more than SAP, except in hepatic

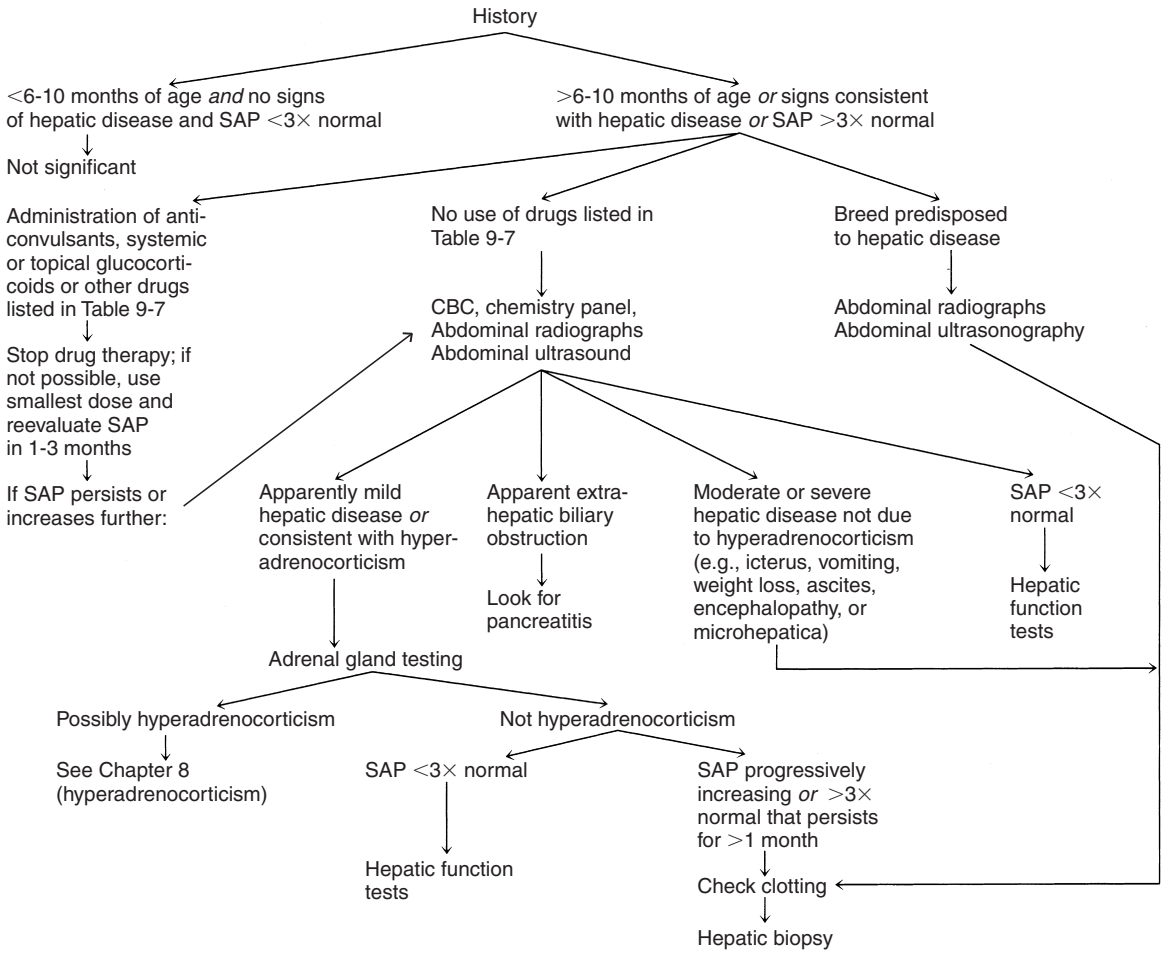

FIGURE 9-8. Diagnostic approach to increased serum alkaline phosphatase (SAP) in dogs. *CBC,* Complete blood count.

lipidosis (where classically the SAP is usually quite high, but GGT values show only a mild [or no] increase). GGT does not tend to increase after acute hepatic necrosis, as does SAP. Increased GGT should be pursued as for increased SAP (see Figure 9-8). Increased GGT may suggest pancreatitis obstructing the bile duct, as for SAP.

Causes of Increased Urine GGT • Increased 24-hour urinary excretion of GGT can be caused by various nephrotoxins (e.g., gentamicin).

LACTIC DEHYDROGENASE

Rare Indications

Disadvantages • Lack of specificity. The lactic dehydrogenase (LDH) test is *not recommended.*

Analysis • Measured in serum, heparinized plasma, or cerebrospinal fluid (CSF) by spectrophotometric methods.

Normal Values and Danger Values • Same as for ALT and SAP.

Causes of Decreased LDH • Not significant.

Causes of Increased LDH • LDH is found in so many body tissues that serum LDH is of questionable diagnostic value. Inexplicable increases of small to great magnitude are not uncommon.

SULFOBROMOPHTHALEIN RETENTION

Rare Indications • In general, sulfobromophthalein (BSP) dye retention is rarely used

anymore, except by research laboratories; it is *not recommended*.

Analysis • See prior editions.

INDOCYANINE GREEN

Rare Indications • Same as for BSP. In general, indocyanine green (ICG) is *not recommended*.

Analysis • See prior editions.

BILE ACIDS

Frequent Indications • Suspected occult hepatic disease, chronic weight loss, abnormal CNS signs, icterus, hepatomegaly, and microhepatia; also to monitor hepatic function in patients with known hepatic diseases. Because of the ease of this test compared with BSP, ICG, and ammonia, serum bile acids are used routinely as a screening test for hepatic dysfunction.

Advantages • Ease of use, few extrahepatic factors affect it.

Disadvantages • Does not reliably distinguish among different hepatobiliary diseases. Values may change enough from day to day that it can be difficult to use the serum bile acid concentrations to determine if a change in hepatic function has or is occurring.

Analysis • Measured in serum by either a direct enzymatic method that quantifies total serum 3-alpha-hydroxylated bile acids or uses an RIA procedure that measures specific bile acids. It is important that a validated assay for dogs and cats be used because some methods are not accurate. Values for enzymatic and RIA procedures cannot be compared.

Maximum information is obtained by determining a 12-hour fasting preprandial and 2-hour postprandial concentration. Dogs and cats should be fed canned food containing moderate fat content, causing the gallbladder to contract. Preprandial and postprandial concentrations together improve the sensitivity of the test, making it more sensitive than other function tests (e.g., resting serum ammonia concentrations).

Normal Values • Because of different techniques and assays (μmol/L or μg/ml), normal values must be established for each laboratory.

Danger Values • None.

Artifacts • Very increased serum dehydrogenase activities may require modification of the spectrophotometric technique. Severe lipemia (i.e., chylomicronemia) and hemolysis may falsely decrease bile acid measurements, and hypertriglyceridemia may falsely increase concentrations when spectrophotometric techniques are used, but they do not affect RIA. This test is not indicated in icteric patients.

Drug Therapy and Other Factors That May Alter Serum Bile Acid Concentration • Cholestyramine lowers serum concentrations by binding to bile acids in the intestinal lumen, preventing their reabsorption. Ursodeoxycholic acid (a synthetic bile acid) therapy may increase total serum bile acid concentrations. Resection of the ileum (the principal site of bile acid reabsorption), severe ileal disease, or cholecystectomy may also cause serum bile acids to inaccurately reflect hepatic function. Prolonged anorexia (>1 to 2 days) may cause fasting serum bile acid concentrations to be less than would be found if the patient were eating normally. Intestinal hypomotility may cause the 2-hour postprandial sample to be a less sensitive indicator of hepatic disease because of failure to deliver the bile acids to the ileum in a timely fashion. ARE may increase total serum bile acid concentrations because of bacterial deconjugation of bile acids with subsequent increased small intestinal uptake. Hepatic insufficiency does not decrease serum bile acid concentrations.

Causes of Decreased Serum Bile Acid Concentration • Delayed gastric emptying, rapid intestinal transit, malabsorption disorders, and ileal resection may cause subnormal values. With ARE, total measurable moieties may or may not decrease, but it is expected that unconjugated serum bile acid concentrations may increase.

Causes of Increased Serum Bile Acid Concentration • Serum bile acid concentrations are increased because of hepatocellular disease, cholestatic disease, or portosystemic shunting. When both fasting and 2-hour

postprandial serum bile acid levels are determined, the sensitivity of these tests becomes greater than with other hepatic function tests. Because of the ease of performing and wide availability of the test, it has replaced other clinical hepatic function tests. Serum bile acids offer no additional information in icteric patients with hepatic or extrahepatic biliary tract disease. In nonicteric patients suspected of having hepatic disease, serum bile acids are a good screening test to support further diagnostic evaluations. Not all patients with hepatic disease have increased serum bile acid concentrations, and the relative increase in bile acids is not diagnostic for the type of disease or the prognosis. Reported fasting serum bile acids that are increased greater than 20 μmol/L or postprandial values of greater than 25 μmol/L suggest significant hepatic disease or portosystemic shunting and dictate further hepatic evaluation and possibly hepatic biopsy. Generally, preprandial and postprandial bile acids are determined simultaneously; however, if only fasted values are determined and found to be normal, postprandial measurements are required. The magnitude of the rise or the percent increase from preprandial to postprandial values does not imply a specific diagnosis or prognosis. Most animals with chronic hepatitis, marked hepatic necrosis, cholestasis, and hepatic neoplasia have abnormal values. Bile acids are usually not markedly altered by secondary hepatic disease from nonhepatic disorders or with glucocorticoid or anticonvulsant therapy; however, in rare cases they may be.

Increased serum bile acids are possibly the most sensitive biochemical indicator of congenital portosystemic shunts. Almost all animals with congenital portal vascular anomalies have increased postprandial bile acids; some of the highest concentrations occur in these cases.

AMMONIA AND AMMONIA TOLERANCE TESTING (ATT)

Rare Indications • Same as for bile acids (i.e., when a sensitive function test is needed to prove hepatic disease in an animal in which easier tests do not allow diagnosis).

Advantages • Good sensitivity and specificity.

Disadvantages • Procedural requirements for submitting the samples, and the likelihood of vomiting or CNS signs with ATT.

Analysis • Measured in blood, serum, plasma (heparinized is recommended), CSF, or urine by enzymatic, selective electrode, dry reagent, and resin absorption methods. There does not appear to be any advantage of arterial over venous blood. Blood must be drawn into an ice-chilled tube, which is stoppered tightly after filling, immediately put back on ice, and promptly taken to the laboratory. A control sample should be taken at the same time using the same technique. The test must be performed within 20 minutes, or the plasma must be frozen at –20° C, which stabilizes the ammonia concentration for at least 2 days. If an ATT is to be performed, samples for ammonia determination should be taken before and 30 or 45 minutes after administration of NH_4Cl, 100 mg/kg of body weight. The NH_4Cl may be administered orally as a solution in 20 to 50 ml of water, as a 5% solution, as a dry powder in gelatin capsules, or rectally as a 5% solution. The use of orally administered gelatin capsules is the easiest and the least likely to result in expulsion (i.e., vomiting or defecation of the NH_4Cl).

Warning: Administration of NH_4Cl to patients with increased resting blood ammonia concentrations may cause encephalopathy. The clinician should not perform ATT if the patient is showing obvious encephalopathic signs. Lack of obvious encephalopathic signs does not guarantee that blood ammonia levels are normal.

Normal Values • Resting ammonia: dogs, 45 to 120 μg/dl; cats, 30 to 100 μg/dl. ATT, ammonia at 30 minutes: dogs, minimal change from normal values; cats, no change from normal values.

Danger Values • Dogs, greater than 1000 μg/dl (hepatic encephalopathy may be imminent, although poor correlation exists between clinical signs of encephalopathy and plasma ammonia concentrations); cats, unknown.

Artifacts • Falsely increased: allowing the blood to stand, strenuous exercise. See Introduction to Serum Chemistries.

Drug Therapy That May Alter Ammonia • Decreased serum ammonia may be the result of intestinal antibiotics (e.g., aminoglycosides), lactulose, *Lactobacillus acidophilus* cultures, enemas, and diphenhydramine. Increased serum ammonia may be the result of valproic acid, asparaginase, narcotics,

diuretics causing hypokalemia or alkalosis, hyperalimentation, ammonium salts, and high-protein meals (including blood from spontaneous GI bleeding).

Causes of Hypoammonemia • Not significant.

Causes of Hyperammonemia • Urea cycle disorders (extremely rare) and hepatic insufficiency (especially congenital or acquired portosystemic shunting). Resting blood ammonia concentrations are probably less sensitive than fasting serum bile acids in detecting hepatic dysfunction, whereas the ATT is possibly as sensitive as preprandial and postprandial bile acids in detecting portosystemic shunting. A significantly increased fasting blood ammonia concentration renders an ATT unnecessary. Clinical signs are not well correlated with blood ammonia concentrations. An abnormal ATT result or resting ammonia value in a patient with hepatic disease is generally an indication for hepatic biopsy or a portogram. Rarely, plasma ammonia is increased because of urinary tract obstruction, especially if complicated by infection with urease-producing bacteria. Some young dogs (notably Scottish deerhounds in Great Britain) have been found to have elevated resting blood ammonia values that spontaneously return to normal as the dog ages. Therefore, caution must be used when diagnosing congenital portosystemic shunting in at least some breeds solely by evaluating the resting serum ammonia concentration.

CHOLESTEROL

See Chapter 8.

WEIGHT LOSS OR ANOREXIA OF UNKNOWN CAUSE

Weight loss has many causes (Table 9-11). Concurrent problems with fewer potential causes (e.g., regurgitation, vomiting, diarrhea, icterus) should be considered first. If a patient had a reasonable appetite when weight loss began, major differential diagnoses are intestinal disease, maldigestion, increased use of calories (e.g., hyperthyroidism, lactation), or increased loss of calories (e.g., diabetes mellitus). If no other identifiable problems (other than weight loss or anorexia) can be pursued, a systematic search is indicated (Figure 9-9). One should first preclude as many causes as possible with the history and physical examination (i.e., lack of food, calorie-deficient food, inability to eat, regurgitation, vomiting and diarrhea). Next, extensive clinicopathologic screening is indicated. Imaging is considered an extension of the physical examination, and abdominal and thoracic radiographs are appropriate. Thoracic radiographs may be very revealing, even if a patient does not have coughing or abnormal lung sounds. Abdominal ultrasonography is particularly desirable and often more useful than radiographs if the operator is accomplished. If laboratory or radiographic abnormalities are not present or are unconvincing, one may repeat the tests at 1 to 3 week intervals, depending on the clinical condition of the patient, or immediately proceed to function tests, biopsies, or both. Certain hepatic and adrenal gland diseases may require such function tests. It is noteworthy that severe gastric or intestinal disease may cause anorexia or severe weight loss without vomiting or diarrhea.

Gastroduodenoscopy and ileoscopy plus biopsy are reasonable in patients with severe weight loss of unknown cause. Some cases with gastric neoplasia may present only for anorexia and weight loss. Clinicians without

TABLE 9-11. Major Causes of Weight Loss in Dogs and Cats

Calorie-Deficient Food or No Food
Failure or Refusal to Eat
Dysphagia
Oral lesion
Anorexia for any reason
Regurgitation
Pharyngeal or esophageal disease
Vomiting (see Table 9-2)
Maldigestion
Exocrine pancreatic insufficiency (EPI) (does not always cause diarrhea)
Intestinal Malabsorption (Does not always cause diarrhea)
Malassimilation
Hepatic failure
Cardiac failure
Diabetes mellitus
Uremia
Cancer cachexia syndrome
Hypoadrenocorticism
Excessive Use or Loss of Calories
Hyperthyroidism
Excessive demand for calories because of environment or exertion
Lactation
Muscle Wasting
Myopathy
Neuropathy

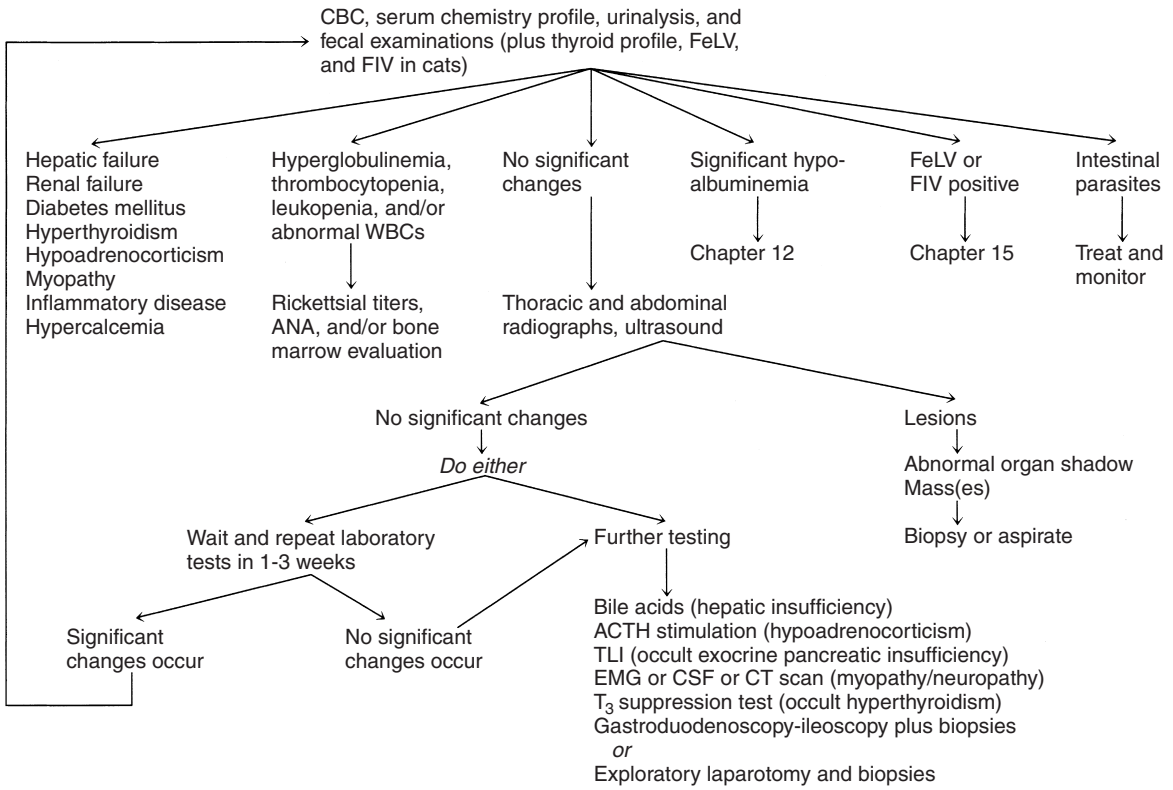

FIGURE 9-9. Diagnostic approach to chronic weight loss in dogs and cats when no other abnormalities are found on history or physical examination and the animal is not ingesting adequate calories (see Table 9-11). *ACTH,* Adrenocorticotropic hormone; *ANA,* antinuclear antibodies; *CBC,* complete blood count; *CSF,* cerebrospinal fluid; *EMG,* electromyogram; *FeLV,* feline leukemia virus; *FIV,* feline immunodeficiency virus; *TLI,* trypsin-like immunore-activity; *WBC,* white blood cell.

access to endoscopic equipment may consider exploratory laparotomy. If surgery is performed, gastric, duodenal, jejunal, ileal, mesenteric lymph node, and hepatic biopsies are usually appropriate, regardless of a normal gross appearance of the organs. In cats, the pancreas should also be biopsied.

Cancer cachexia can be particularly difficult to diagnose. It is a poorly defined, multifaceted syndrome that may involve loss of taste, malabsorption, increased metabolism with energy wasting, and other mechanisms. Almost any tumor can cause cancer cachexia, and no consistent laboratory findings exist. The causative cancer may be large or small, focal or diffuse; lymphomas and carcinomas are probably the most common causes.

Anorexia of unknown cause is similar to weight loss in being difficult to evaluate if no other identifiable abnormalities are seen. The diagnostic approach is similar to that for chronic weight loss (see Figure 9-9; Table 9-12).

TABLE 9-12. Categories of Diseases That Cause Anorexia

Psychologic (especially cats)
Inability to smell food
Dysphagia (especially when it causes pain)
Inflammation
Because of an etiologic agent
Because of immune-mediated disease
Because of neoplasia
Because of necrosis
Because of drugs
Alimentary and abdominal disease (especially that which causes nausea or abdominal pain)
Neoplasia
Because of the neoplasia itself
Because of secondary bacterial infection when the neoplasia impairs natural defense mechanisms
Toxins
Exogenous (various ones)
Endogenous (e.g., renal failure, hepatic failure)
Endocrine disease
Hypoadrenocorticism
Hyperthyroidism
Central nervous system (CNS) disease
Primary
Secondary

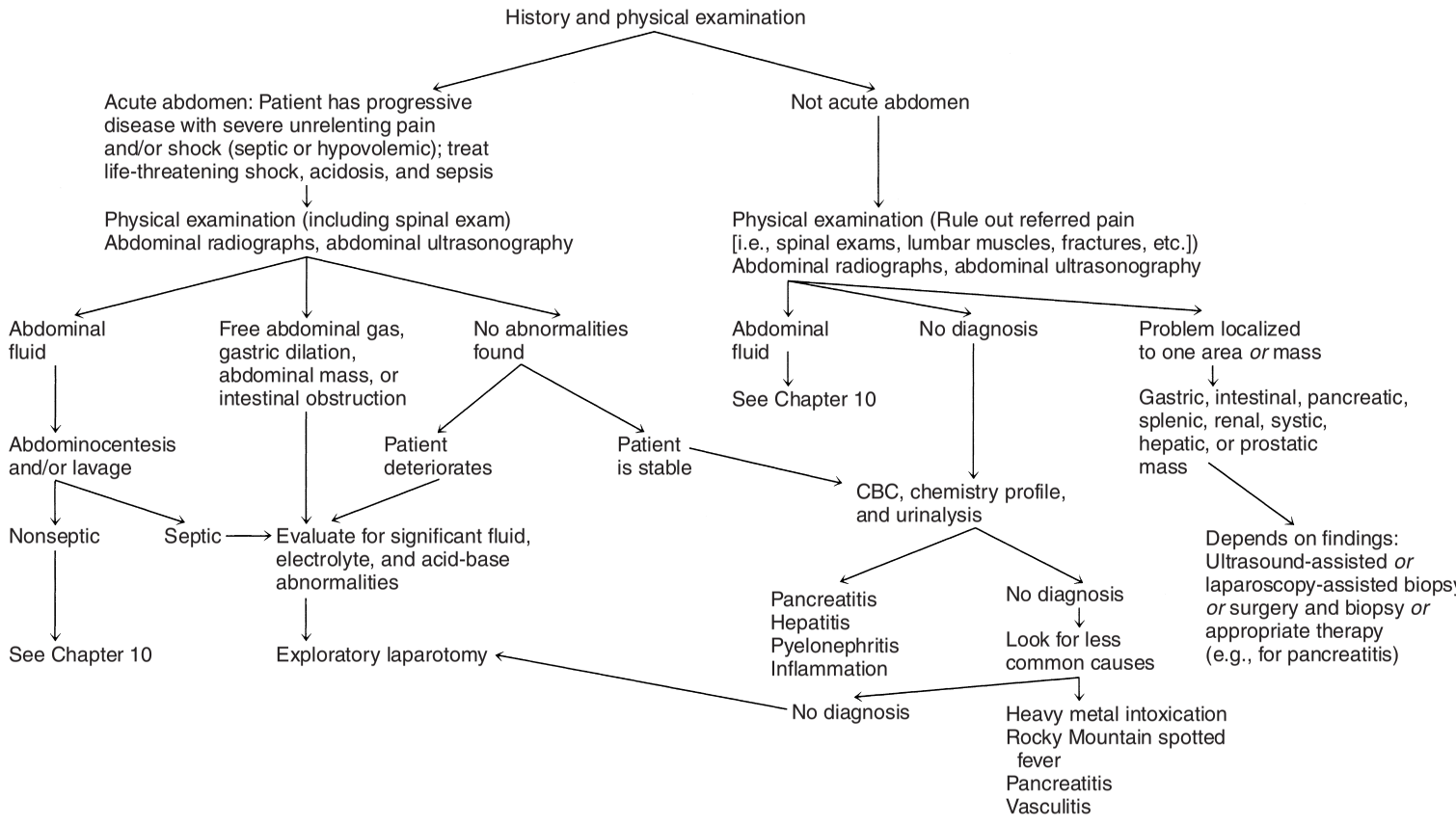

FIGURE 9-10. Diagnostic approach to abdominal pain in the dog and cat. *CBC,* Complete blood count.

Anorexia can be divided into three categories: (1) pseudoanorexia associated with inability to eat (oral, pharyngeal, or esophageal disease), (2) primary anorexia (rare) associated with a primary CNS disorder, and (3) secondary anorexia (the most common), which is the result of systemic or metabolic disease.

If necessary, one may elect a therapeutic trial to treat for a suspected problem in a patient in whom a diagnosis cannot be made. It is vital that one design such therapy so that it is safe and extremely likely to succeed if the presumptive disease is present. Then, if the trial fails, one may rule out that disease and go on to treat for something else. To do this, the clinician must be sure that the dose and duration of the treatment is sufficient.

ABDOMINAL PAIN

History, physical examination, radiographs, and ultrasonography are the initial tools in diagnosing the cause of abdominal pain (Figure 9-10). Extra-abdominal diseases such as spinal problems and patients predisposed to nonsurgical diseases (e.g., pancreatitis) must be identified early.

In patients with severe, progressive, acute abdomen (severe unrelenting pain or shock or stupor in a deteriorating patient), surgery is often indicated as soon as fluid, electrolyte, and acid-base status are acceptable for anesthesia. Imaging is desirable, but extensive laboratory testing is unlikely to identify the more common causes of acute abdomen (e.g., intestinal obstruction, gastric dilation and volvulus, peritonitis, organ ischemia, tumor, sepsis, or bleeding) and usually only delays surgical resolution of disease. Abdominal exploration offers a good chance for definitive diagnosis plus resolution of the disease process.

NOTE: These maladies do not always present as surgical emergencies.

If a patient is not in severe pain and the disease is not progressing rapidly, one must differentiate between problems that ultimately necessitate surgery and those that usually do not (e.g., pancreatitis, hepatitis, cholecystitis, upper urinary tract infection, prostatitis, pansteatitis, heavy metal intoxication, Rocky Mountain spotted fever [RMSF]). Abdominal ultrasonography is useful to examine the liver, spleen, pancreas, kidneys, and prostate, as well as to detect peritoneal fluid. If abdominal fluid is present, abdominocentesis or abdominal lavage with cytologic analysis is indicated. If these procedures are not revealing and the problem continues, exploratory surgery may be necessary. Contrast radiographs are rarely useful because thorough abdominal exploration should diagnose almost anything they reveal; finding an abnormality on radiographs simply is an indication for surgery. In rare situations the exhibited abdominal pain may be referred from other causes such as pulmonary disease or disk disease.

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10

Sharon A. Center

Fluid Accumulation Disorders

DIAGNOSTIC APPROACH

Effusion describes the inappropriate accumulation of fluid in a body "potential" or "third" space outside of vascular or lymphatic conduits and visceral structures. Physical examination often reveals effusions. Estimation of skin turgor and thickness of lip folds, conjunctiva, and tarsal webs detects localized edema or whole-body edema (i.e., anasarca). Some effusions can be difficult to recognize without diagnostic imaging (radiographic or ultrasonographic). Abdominal effusion can be missed during palpation (balloting a fluid wave has poor predictive value). Small volumes of pleural fluid usually escape physical detection. In almost all cases, analysis of the fluid causing the effusion is crucial to proper categorization and determination of cause.

FLUID COLLECTION TECHNIQUES

Collection of Fluid • The puncture site is prepared as for aseptic surgery. A 1-inch, 23- to 20-gauge needle, over-the-needle Teflon catheter, or butterfly needle–catheter is recommended. Fluid analysis usually requires 3 to 5 ml. If ultrasound guidance is used, the clinician should prevent sample contamination with ultrasound gel that induces artifacts (i.e., blue-appearing smudges on Diff-Quik or Wright's Giemsa-stained preparations).

Abdominocentesis • The abdomen is palpated immediately before abdominocentesis to avoid lacerating visceral structures. The urinary bladder should be emptied before abdominocentesis, particularly when 14-gauge catheters are used. In patients with tense abdominal distention, the abdomen is punctured laterally (to avoid gravitational ventral midline seroma formation). If septic peritonitis is suspected but unproven with routine abdominocentesis, a four-quadrant tap is performed. When fluid is difficult to collect, ultrasonographic guidance is helpful. Usually a 22- to 20-gauge, 1-inch needle or Teflon catheter is attached to extension tubing. Extension tube length helps avoid visceral laceration should the patient move during the procedure.

Alternatively, a butterfly catheter is used. If the effusion is difficult to sample, a 14-gauge Teflon catheter is used to puncture the

abdomen, and a closed-ended polypropylene Tomcat catheter inserted through its lumen. The Teflon catheter provides a "sterile stent" through which the Tomcat catheter may be manipulated as the patient's position is altered.

Abdominal Lavage • Sterile warmed physiologic saline (20 ml/kg) is administered intraperitoneally over 5 to 10 minutes through extension tubing and a Teflon catheter. The abdomen is massaged or the animal moved about for several minutes to mix this fluid with that trapped within omental recesses and abdominal gutters. Lavage fluid is subsequently aspirated and analyzed. Dogs normally have less than 500 white blood cells (WBCs)/µl. Mild leukocytosis occurs after recent abdominal trauma or surgery. Diagnostic guidelines for interpretation of abdominal lavage fluid are listed in Table 10-1. Iatrogenic injury and bacterial contamination are possible consequences of lavage. Dilutional influences on the collected fluid can create diagnostic confusion.

Thoracocentesis • It is usually performed in the seventh or eight intercostal space at the level of the costochondral junction; however, relevant imaging studies may better guide sample collection. The needle penetrates the middle of the intercostal space, avoiding the caudal rib margin where the nerves and vessels are located. Harvesting fluid is optimal with the animal standing or in sternal recumbency. A 1-inch, 18- or 20-gauge butterfly catheter connected to a three-way stopcock and a 20- to 35-ml syringe are recommended. During initial needle placement, negative pressure is maintained on the syringe so that advancement of the needle immediately discloses effusion, thus avoiding inadvertent pulmonary puncture or laceration. Repeated centesis should be performed after a local anesthetic block is applied to the puncture site.

TABLE 10-1. Suggested Guidelines for Interpretation of Abdominal Lavage Effusion*

Turbidity	
Clear:	No disease or abdominal injury
Bloody:	Iatrogenic or hemorrhage *Chronic effusion*: serosanguineous
Blood darkens on repeat centesis:	Active hemorrhage: acquire packed cell volume (PCV) for relative change
Turbid:	Cannot clearly read newsprint through fluid: *cytology indicated*
PCV: <5%	Mild hemorrhage
>10%	Significant hemorrhage
WBC: <500/µl	Normal dogs
>1000/µl	Mild to moderate inflammation
>2000/µl	Probable peritonitis: *cytology indicated*
Pancreatic enzymes:	
Lipase or amylase	*If> sera:* pancreatic inflammation, injury, necrosis
Total bilirubin:	*If> sera:* bile spillage or enteric rupture
Creatinine:	*If> sera:* urinary tract rupture, urine spillage
Vegetable fibers:	Enteric rupture or sampled enteric lumen
Mixed bacterial flora:	Enteric rupture, ruptured abscess or sampled enteric lumen

*After instillation of 20 ml/kg and mixing throughout peritoneal cavity.

Pericardiocentesis • The catheter is usually passed through the fifth or sixth right intercostal space (i.e., the cardiac notch between lung lobes). Local anesthetic is injected to the level of the pleura. A 12- to 16-gauge, 4- to 6-inch over-the-needle Teflon catheter with two or three extra holes aseptically snipped in the lateral aspect is used. Extension tubing and a three-way stopcock are necessary in medium- and large-sized dogs. The site of thoracic penetration is surgically prepared, and a small incision is made to facilitate movement of the catheter through the dermis. An electrocardiogram (ECG) is simultaneously recorded while the catheter is advanced; touching the myocardium elicits premature ventricular beats. Entrance into the pericardium may require an acute thrust. The catheter is slipped over the needle into the pericardial sac, and the needle discarded. If bloody fluid is extracted, the clinician should immediately compare packed cell volume (PCV), number of platelets, total solid concentration, supernatant color, and activated clotting time between fluid and peripheral blood. Comparison avoids inadvertent removal of large volumes of intracardiac blood.

Collection of Edema Fluid • A 22- to 25-gauge needle is gently introduced into affected tissues. Fluid often drains spontaneously by gravity but can be assisted by gentle massage or aspiration.

CHARACTERIZATION OF FLUID

Fluid Analysis • Collected fluids should be analyzed immediately to permit its

characterization and determine whether further testing (e.g., bacterial culture) is appropriate. Three to 5 ml aliquots of fluid should be stored in an ethylenediaminetetraacetic acid (EDTA) tube and sterile clot tube for cytology and chemical analysis, respectively. A separate sample for culture is stored in a sterile clot tube, a culturette containing transport media, or broth culture media. If only a few drops of fluid are collected, cytology has first priority. Cultures can be taken from the needle hub with a microtip culturette, or the needle and syringe can be washed with culture broth. PCV, total protein, and appearance of microcentrifuged supernatant of bloody effusions should be compared with peripheral blood. Physicochemical and cytologic assessment of effusions usually permits their classification into one of several categories (Table 10-2).

Physical Assessment of an Effusion • Turbid fluids contain cells or lipids. Chylous effusions are usually white, pink, or opalescent, and the supernatant turbid. Red-tinged or maroon fluid indicates red blood cells (RBCs) or free hemoglobin. Blood-tinged fluids must be centrifuged to determine their PCV relative to systemic PCV and to permit supernatant evaluation. RBCs often accumulate in effusions secondary to inflammation or vascular congestion, causing a PCV less than or equal to 8%. If the PCV is more similar to systemic blood and supernatant is clear, acute hemorrhage or iatrogenic sample contamination is likely. PCV may be artifactually lowered by hemolysis caused by fluids with very high or low tonicities, by freezing and thawing a sample, high lipid concentrations, and trauma (i.e., forced sample injection into vacutainer). If supernatant is maroon, hemolysis is probable. Erythrophagocytosis (i.e., RBCs engulfed by macrophages) and macrophages containing hemosiderin (i.e., siderocytes) occur when blood has contaminated an effusion for longer than a day. Erythrophagocytosis can also develop in fluid stored longer than a few hours. Chronically blood-contaminated samples lack platelets, and supernatant appears xanthochromic (yellow-tinged) after centrifugation. Xanthochromia reflects the presence of bilirubin pigments. A jaundiced animal has yellow effusions. Bile peritonitis usually produces a brown-green or dark yellow-green effusion containing both free and engulfed bilirubin crystals. Septic effusions may have a foul smell caused by anaerobic bacteria.

Cell Counts and Cytology • Total and differential nucleated cell counts are performed on anticoagulated, noncentrifuged fluid. Total cell counts usually require a hemocytometer because of debris that impairs function of automated counters. Very small volume samples may yield falsely low cell counts because of dilution of fluid with anticoagulant. Poor sample mixing, sample contamination, prolonged storage, and medical therapy may each influence cell counts. Differential counts are best performed from concentrated cellular components. However, smears of unconcentrated fluid allow estimation of cell numbers when cell counts are greater than 1000/μl. At least six slides of collected fluid should immediately be made. Slides are rapidly air-dried and stained using a modified Wright's stain such as Diff-Quik. If bacteria are visible, Gram's stain is applied. If the fluid appears relatively acellular, a portion should be centrifuged and smears made of the sediment as soon as possible. Cytospin centrifugation provides the best cellular morphology. Cytology smears should be rapidly air-dried to preserve cell morphology. The clinician should remain alert for microorganism-contaminated stains, post-sampling degeneration of neutrophils associated with prolonged storage in EDTA or saline, or cell changes induced by exposure to urine or bile.

DISTINGUISHING DIFFERENT TYPES OF EFFUSIONS

Pure Transudates

Pure transudates are associated with low oncotic pressure, increased hydrostatic pressure, and sometimes-increased vascular permeability. These are poorly cellular (i.e., < 1000 cells/μl), have total solids less than 2.5 g/dl, and a specific gravity less than 1.017. Classic examples are abdominal effusions caused by hypoalbuminemia resulting from hepatic insufficiency, protein-losing nephropathy (PLN), and protein-losing enteropathy (PLE).

Modified Transudates

Modified transudates are associated with a higher total solids concentration than pure transudates (generally 2.5 g/dl), a specific gravity less than 1.017, and moderate cellularity. Mesothelial cells are usually plentiful, and the other cellular components highly variable.

TABLE 10-2. Characteristics of Selected Types of Effusions

	TRANSUDATES			EXUDATES			
	PURE TRANSUDATE	**MODIFIED TRANSUDATE**	**HEMORRHAGIC EFFUSION**	**NONSEPTIC EXUDATE**	**SEPTIC EXUDATE**	**BILIOUS EFFUSION**	**CHYLOUS EFFUSION**
Color	Clear Watery	Serous Serosanguineous	Bloody	Serosanguineous	Purulent, creamy Serosanguineous	Brown/green Dark yellow/green	Milky/white/pink Opalescent
Turbidity	Clear	Clear to cloudy	Opaque	Cloudy	Cloudy/flocculent	Opaque	Opaque
Total solids (g/dl)	<2.5	2.5–5.0	>3.0	>3.0	>3.0	>3.0	>2.5
Specific gravity	<1.017	1.017–1.025	>1.025	>1.025	>1.025	>1.025	>1.018
Nucleated cells/μl	<1000	500–10,000	>1000	>5000	>5000	>5000	Variable
Differential	Mononuclear cells (mesothelial cells, lymphocytes, macrophages)	Mesothelial Macrophages Neutrophils (nondegenerate) RBCs (few) Lymphocytes	Similar to blood Neutrophils (variable, nondegenerate) Lymphocytes (few) Macrophages (erythrophago-cytosis)	Neutrophils (nondegenerate) Macrophages (phagocytized debris) RBCs (variable) Mesothelial (increased in chronic) ± Neoplastic cells	Neutrophils (degenerate, phagocytized bacteria) Macrophages (phagocytized bacteria) Mesothelial (variable) RBC (variable)	Neutrophils (predominate in acute) Macrophages (phagocytized and free bilirubin crystals: brown-granular material) Lymphocytes (few)	Lymphocytes (predominate early) Neutrophils (increase in chronic) Mesothelial cells (variable)
Bacteria	No	No	No	No	May see bacteria	±	Rare
Lipid	No	No	No	No	No	No	High triglycerides (fluid > sera) Cholesterol (fluid < sera) Stain positive with Sudan III or oil Red O

Hemorrhagic Effusions

Hemorrhagic effusions appear bloody, have a measurable hematocrit, and have total solids greater than 3.0 g/dl. If chronic, the supernatant evidences hemolysis or xanthochromia, and cytologic inspection reveals erythrophagocytosis, siderocytes, and a lack of platelets. These effusions do not clot. Platelets appear only when bleeding has occurred within approximately 1 hour of sampling. Peracute or iatrogenic hemorrhage has no erythrophagocytosis or siderocytes, supernatant is clear, and platelets are expected.

Exudates

Exudates are characterized by high total solid concentration (i.e., >3.0 g/dl), high specific gravity (i.e., >1.025), and increased cellularity dominated by neutrophils and macrophages (i.e., >5000 cells/μl). These effusions may be septic or nonseptic. Inflammatory, necrotizing, infectious, or malignant disorders can be responsible. Exudative effusions should be cultured aerobically and anaerobically. One should immediately determine cytologically whether an effusion is exudative or not to prioritize the importance of culture.

Bilious Effusions

Bilious effusions contain intracellular and extracellular bilirubin crystals (yellow, golden, or brownish material). Large numbers of neutrophils that may be highly segmented and reactive mesothelial cells are common. Bacteria may be visible. Comparing fluid bilirubin concentration to peripheral blood values (the former will be higher) is diagnostic. Jaundiced animals develop yellow-colored effusions.

Chylous Effusions

Chylous effusions usually have total solid concentrations greater than 2.5 g/dl; specific gravity greater than 1.018; high numbers of neutrophils, lymphocytes, or both; and a triglyceride concentration exceeding peripheral blood values. The fluid:serum triglyceride ratio is greater than 2 to 3:1 and commonly exceeds 10:1. Effusion cholesterol concentration is typically less than peripheral blood cholesterol. When centrifuged, chylous effusions have lactescent or opalescent supernatants. A buoyant, triglyceride-rich chylomicron layer accumulates at the surface upon sample refrigeration. A qualitative test for high triglyceride content involves incubation of the effusion pretreated with 1 to 2 drops of 1 N sodium hydroxide, with an equal volume of ether. Ether-soluble triglycerides rise to the top of the tube as a white band. Alternatively a wet mount of fluid may be stained with oil red O or Sudan black and subsequently evaluated for fat droplets (i.e., chylomicrons) (see Color Plate 5B).

Pseudochylous Effusions

Pseudochylous effusions described in the older veterinary literature are extremely rare; they have high cholesterol and low triglyceride concentrations relative to peripheral blood.

Malignant Effusions

Malignant effusions are usually modified transudates or exudates, often blood-tinged and xanthochromic, and are diagnosed by finding neoplastic cells. *Caution:* Reactive mesothelial cells are often misinterpreted as malignant (e.g., binucleated cells, signet ring–shaped cells similar to carcinoma cells, high nuclear:cytoplasmic ratio, large and variably sized nucleus and nucleoli). Immunohistochemical distinction of cell type has become an important diagnostic evaluation in human medicine and is just developing in veterinary medicine. So far, however, no tumor marker has a spectrum wide enough to detect all types of malignancies and cannot consistently differentiate between reactive or hyperplastic mesothelial cells and adenocarcinoma cells that can resemble one another. Hemangiosarcomas commonly cause malignant effusion in dogs. These effusions contain large numbers of foamy macrophages, mesothelial cells, and erythrophagocytes; are xanthochromic; lack platelets; and are usually accompanied by acanthocytes and schistocytes (see Chapters 2 and 3) in peripheral blood. Often they produce hemorrhagic effusions without evidence of neoplasia. Carcinomatosis (i.e., miliary tumors implanted on peritoneal or pleural surfaces) frequently causes effusions. Radiography discloses ill-defined serosal margins, and ultrasonography discerns small fluid volume. Some lesions may be large enough for aspiration biopsy. These effusions have a high specific gravity, large amounts of protein, and so many RBCs that they are occasionally classified as hemorrhagic.

Cytology may reveal neoplastic cells; however, *it is easy to confuse reactive mesothelial cells with neoplastic cells*. Particularly vexing is diagnosis of mesotheliomas, which often requires tissue sampling for definitive diagnosis.

Eosinophilic Effusions

Eosinophilic effusions contain greater than 10% eosinophils. Approximately 50% of these effusions are modified transudates and the remainder nonseptic exudates. These effusions cannot be predicted from circulating eosinophil counts. Canine eosinophilic pleural effusions may be associated with heartworm disease, disseminated eosinophilic granulomatosis, systemic mastocytosis, interstitial pneumonia, lymphoma, and hemangiosarcoma. Radiographically apparent infiltrates are common. Pneumothorax can precede development of eosinophilic pleural effusion.

SPECIFIC BODY CAVITY EFFUSIONS

Abdominal Effusions

Clinicians need to be familiar with diagnostic considerations in animals with suspected abdominal effusion (Figure 10-1). *Pure transudates* are typically associated with severe hypoalbuminemia (see Chapter 12; Figure 10-2) and are usually caused by PLN, PLE, hepatic failure, protein loss from exudative cutaneous lesions, or repeated body cavity lavage. Anorexia and emaciation alone do not produce hypoalbuminemia severe enough to elicit edema or effusion. Check for pathologic proteinuria (see Chapter 7) and hepatic insufficiency (see the discussion of bile acids in Chapter 9). *Remember:* PLE may occur without signs of enteric disease; enteric biopsy may be needed for diagnosis. Serum cholesterol concentration (see Chapter 8) is helpful; PLE and hepatic failure usually cause hypocholesterolemia, whereas PLN usually causes hypercholesterolemia. Involvement of portal hypertension as a pathomechanism of abdominal effusion can be discerned by measuring the serum albumin-effusion albumin (SA-EA) gradient; values greater than 1.1 suggest portal hypertension. However, considerable overlapping of SA-EA gradients occurs such that values cannot distinguish the definitive underlying cause (liver disease versus no liver disease).

Modified transudates are usually associated with increased venous (capillary) hydrostatic pressure (see Figure 10-2). Concurrent hepatomegaly suggests impaired blood flow at the level of the hepatic venules, vena cava craniad to the diaphragm, pericardium, right atrium, or pulmonary arterial bed. The clinician should look for jugular pulse, pulsus paradoxus, hepatojugular reflex, weak femoral pulses, muffled cardiac sounds, exercise intolerance, and physiologically inappropriate tachycardia, which might indicate pericardial tamponade. *Hepatojugular reflex* is elicited by applying gentle abdominal compression to liver or cranial abdomen for 10 to 15 seconds (increases venous return to the heart) and seeing jugular vein distention or pulsation (indicating reduced right heart function or filling). Hepatomegaly caused by venous congestion may be difficult to palpate because of abdominal distention or patient conformation.

Thoracic radiographs evaluate the shape and size of the cardiac and pericardial silhouette; the tortuosity and filling of the pulmonary arterial bed; and the shape, distention, and position of the vena cava. If cardiomegaly is present, ultrasonographic evaluation differentiates cardiac from pericardial disease. Abdominal ultrasonography (Doppler color flow enhances evaluation) may reveal distended hepatic veins and an exaggerated flow pattern because of outflow obstruction, abdominal masses (i.e., neoplasia, granuloma), or thrombi obstructing portal blood flow. Central venous pressure (CVP) values greater than 8 cm H_2O are suggestive, and values greater than or equal to 14 cm H_2O are diagnostic of right-sided cardiac dysfunction, filling, or impaired flow of blood into the lungs (e.g., pulmonary hypertension, thromboembolism). CVP does not detect venous hypertension caused by disease caudal to the heart and may be hazardous in patients with bleeding tendencies (e.g., hepatic insufficiency, rodenticide toxicity, vasculitis, thrombocytopenia). CVP may be normal in dogs with cor triatriatum dexter (abnormal congenital occlusive webbing within the right atrium). CVP is subject to many mechanical variables (e.g., catheter end position; kinking, folding, occlusion) necessitating CVP interpretation with other diagnostic assessments.

Modified transudates due to chronic hepatic disease can develop before severe hypoalbuminemia occurs if the animal has sinusoidal or venous hypertension. Pooling of albumin in the ascites of these patients promotes hypoalbuminemia. Abdominal ultrasonography usually discloses microhepatia, or irregular lobe margins or altered parenchymal

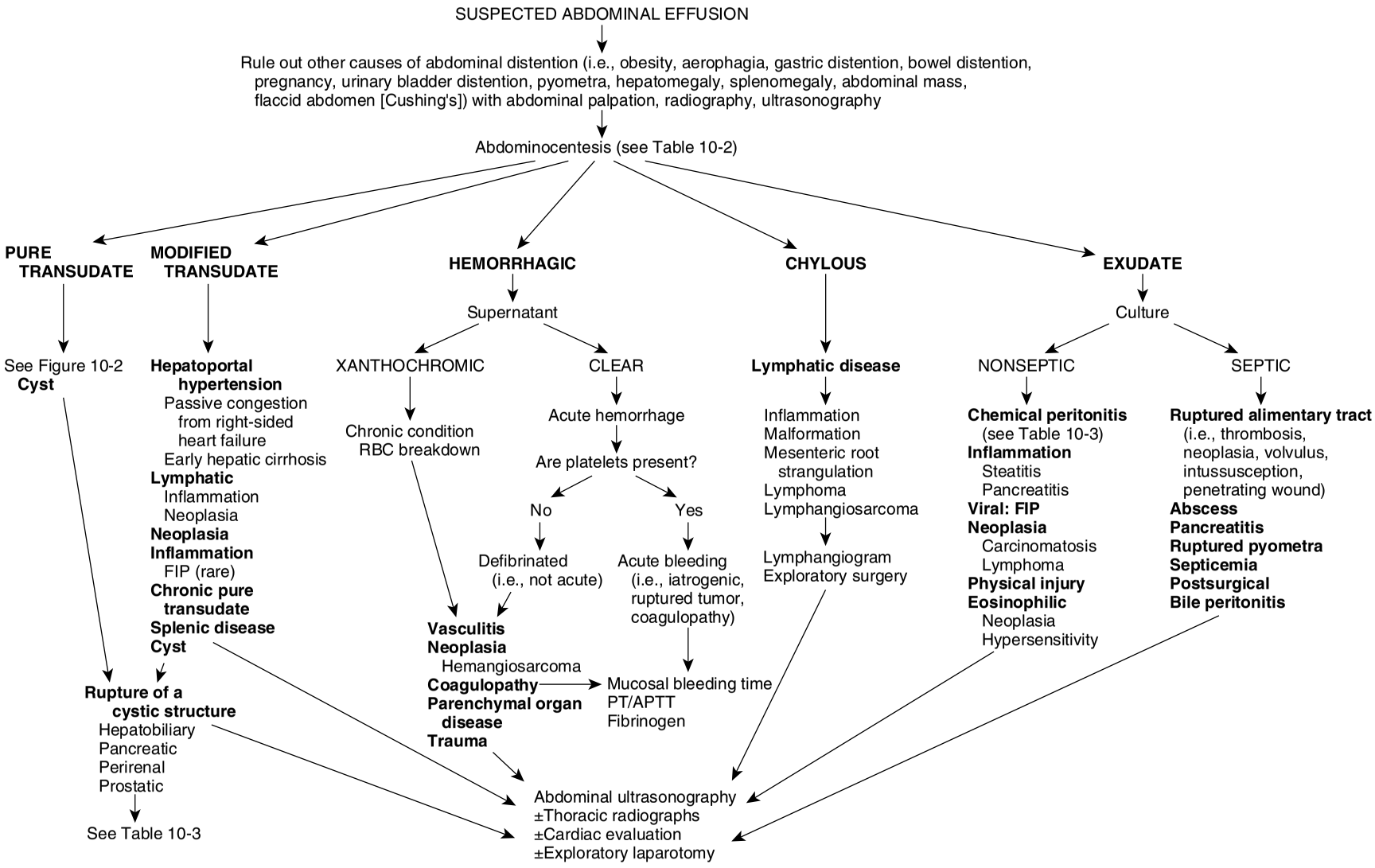

FIGURE 10-1. Diagnostic considerations in animals with suspected abdominal effusion. *APTT*, Activated partial thromboplastin time; *FIP*, feline infectious peritonitis; *PIVKA*, proteins induced by vitamin K absence; *PT*, prothrombin time.

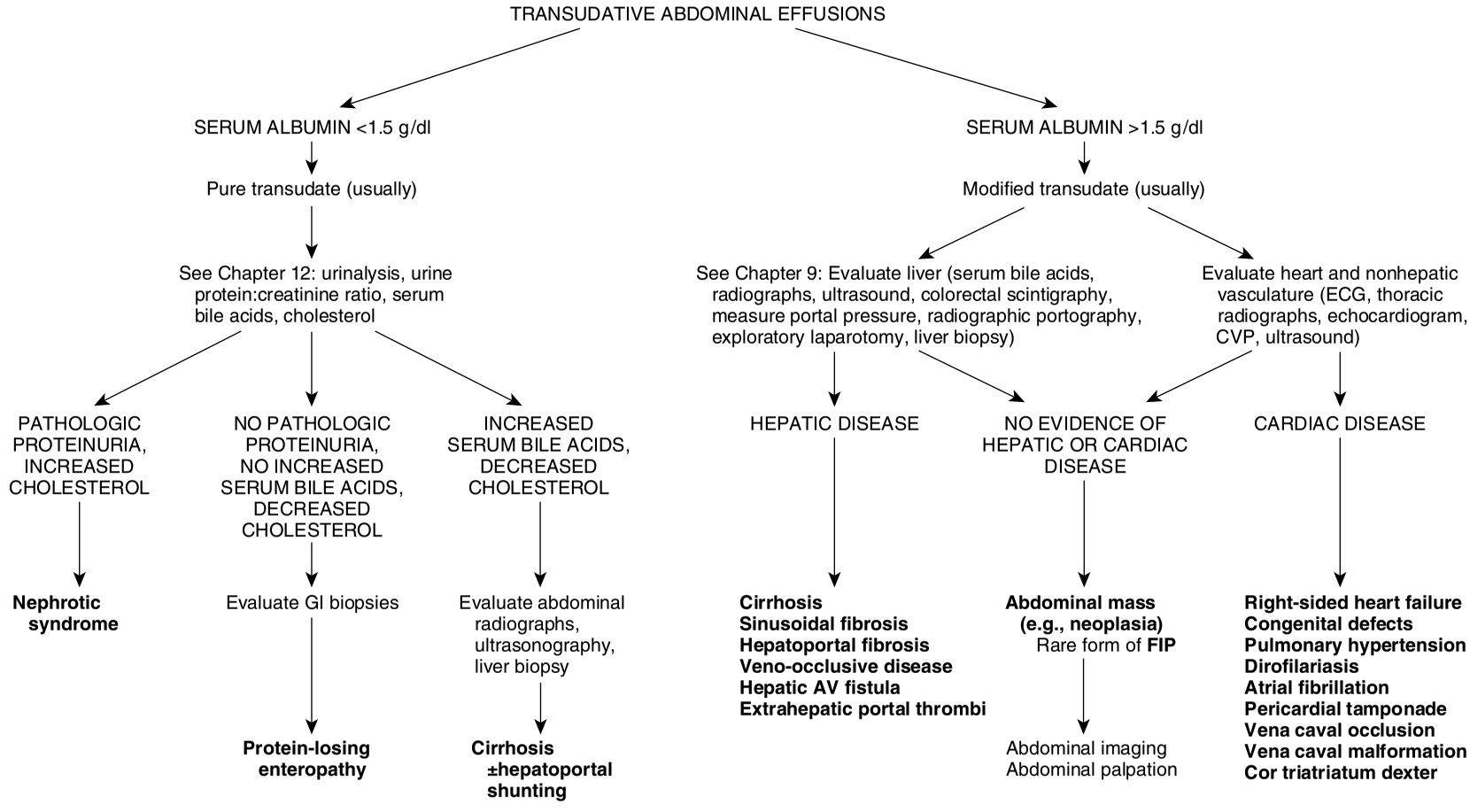

FIGURE 10-2. Diagnostic considerations in animals with abdominal transudates. *AV*, Arteriovenous; *CVP*, central venous pressure; *ECG*, electrocardiogram; *FIP*, feline infectious peritonitis; *GI*, gastrointestinal.

echogenicity, or distended abdominal portal vasculature, or splenic congestion, or tortuous portosystemic shunts, or a combination of these. Modified transudates also develop because of fibrosis in the porta hepatis, neoplasia occluding the portal vasculature, or portal venous thromboembolism. The S:E albumin gradient is greater than or equal to 1.1 in these cases.

Splenic infarction, thromboembolism, or torsion may produce a modified transudate or exudate. Portions or the entire spleen may appear enlarged. Splenic abnormalities are usually discovered by ultrasonographic evaluation with color flow Doppler. Further definition requires contrast portography or exploratory laparotomy. Abdominal masses (i.e., neoplasia, granulomas) may also be responsible for modified transudates; these are usually detected by ultrasonography.

Effusions generally make radiographs less useful than ultrasonography. An effusion may be evacuated and radiographs repeated after drainage to determine hepatic size, the presence of abdominal masses, or changes in visceral positions. Visceral margins remain ill defined, however, because all the fluid cannot be aspirated, and effusions often reaccumulate within hours to days. Repeated fluid removal will further compromise serum albumin concentrations.

Exudates mandate searching for infection. Finding phagocytized organisms in neutrophils or macrophages is definitive but may require careful, tedious inspection of a cytology smear. Plant fibers, enteric debris, or a mixed "fecal flora" are consistent with gut rupture. Degenerate WBCs (see Chapter 16 and Color Plate 4B) suggest infection, although some organisms do not alter neutrophil morphology (e.g., Actinomyces) (see Color Plates 4A to 4C). Degenerate changes may be the result of specimen handling. Recent abdominal trauma (e.g., exploratory laparotomy) causes mild, transient fluid neutrophilia with neutrophilic degeneration. Finding and identifying infecting organisms can be difficult, especially when bacterial numbers are low or bacteria are within a granulomatous reaction or abscess. Certain organisms are notoriously difficult to find (e.g., *Nocardia* and *Actinomyces*). Therefore all exudative effusions should be cultured aerobically and anaerobically (see Chapter 15), and samples should be immediately submitted in sterile clot tubes or transferred to appropriate transport media. If samples in transport media cannot be immediately submitted, they should be refrigerated to slow bacterial growth to avoid media substrate use and microbe death. Finding irrefutable evidence of an infectious organism is almost always an indication for emergency exploratory surgery. Animals with probable abdominal contamination caused by recent surgery, repeated drainage, or fluid sampling are exceptions; in these cases, cultures are submitted and antimicrobial therapy tailored to the identified organisms.

Exudates without cytologic evidence of sepsis necessitate reviewing history for trauma and possible urinary, biliary, or cyst rupture (Table 10-3). Intraabdominal inflammation can persist for weeks after blunt abdominal trauma. Radiography of bony structures sometimes reveals injury. If trauma is unlikely, physical examination is reviewed for other sites of inflammation. In cats, feline infectious peritonitis (FIP) must be considered. Although clinical presentations are variable, most cats with FIP are chronically ill with systemic signs, hyperglobulinemia (i.e., ≥.5 g/dl), and a high-protein abdominal effusion (i.e., >4.5 g/dl). Cytology reveals variable nucleated cell counts with mixed inflammation. Inconsistencies in the signs and clinical pathologic findings in FIP (see Chapter 15) make conclusive diagnosis impossible in cats with atypical features (e.g., long-term survival, modified transudative effusions) without tissue immunohistochemistry. Serologic PCR of coronavirus is not a definitive diagnostic test. Other considerations are summarized in Figure 10-1.

Neoplasia becomes an important differential for modified transudates or exudates after eliminating other differentials. Neoplastic cells are sometimes identified cytologically; however, there are typically few or no exfoliated neoplastic cells found in small volume samples. Cytospin preparation or examination of centrifuged fluid sediment increases the chance of finding malignant cells. Sometimes neoplastic cells are only found after collection and centrifugation of large fluid volumes (e.g., >50 ml). *Remember:* Reactive mesothelial cells resemble carcinoma cells. Ultrasonography can aid in aspiration of mass lesions.

Chylous abdominal effusions usually suggest intestinal lymphangiectasia; lymphoproliferative disease of the gut, mesenteric lymphatics, or lymph nodes; or they may suggest intraabdominal neoplasia "strangulating" the mesenteric root. Vitamin E–responsive steatitis and biliary cirrhosis have accompanied

TABLE 10-3. Characteristics, Causes, and Diagnosis of Chemical Peritonitis

	BILE PERITONITIS	UROABDOMEN	PANCREATITIS	RUPTURED "CYSTS"
Appearance	Golden brown, golden green, serosanguineous, turbid	Light to dark yellow ± serosanguineous, clear (some acute), turbid (chronic)	White, yellow, serosanguineous, turbid	Clear to turbid, pale to yellow, colorless
Causes	Blunt abdominal trauma Necrotizing cholecystitis Cholelithiasis	*Trauma:* avulsed ureter or bladder, ruptured bladder Neoplasia Urolithiasis	Pancreatitis	Perirenal cysts Polycystic renal/ hepatic disease Pancreatic cysts Paraprostatic/ prostatic cysts
Clinical features	Vague abdominal pain Lethargy Pale or acholic feces Increased hepatic enzymes Jaundice (chronicity) Septic peritonitis *Gallbladder:* may be difficult to visualize on ultrasonography	Dehydration Azotemia Anuria/oliguria Abdominal distention Hyponatremia/ hyperkalemia Hyperphosphatemia/ acidosis	Anorexia Vomiting Abdominal pain Lethargy Fever Jaundice Increased hepatic enzymes Increased lipase/ amylase/TLI Increased cholesterol Increased bilirubin Cardiac arrhythmias Pleural effusion Acute renal failure	Vary with underlying tissue involved and severity of lesion
Definitive diagnosis	Free and phagocytized bilirubin crystals Fluid bilirubin greater than serum bilirubin May require ultrasound-directed fluid aspiration or hepatobiliary scintigraphy	Intravenous urogram Retrograde ureterocystography Fluid creatinine greater than serum creatinine	Macrophages contain refractile lipid inclusions Fluid lipase/amylase/TLI greater than serum lipase/amylase/TLI	Ultrasonography Tissue biopsy Cyst aspiration + fluid analysis/ cytology

TLI = trypsin-like immunoreactivity.

feline chyloabdomen (see Figure 10-1). Lymphocytes are the initial cell type found, but with chronicity a neutrophilic inflammation predominates. Repeated aspiration of chylous effusions depletes protein (i.e., chyle contains 1 to 6 g protein/dl), which potentiates subsequent fluid accumulation. Secondary infections are rare because of the bacteriostatic effect of chyle. Animals with chyloabdomen require thoracic radiographs to look for pleural effusion, lymphadenopathy, or metastatic neoplasia. Ultrasonography may reveal mesenteric root masses or mesenteric lymphadenopathy.

Hemoabdomen may be associated with trauma (e.g., ruptured spleen or hepatic parenchyma; avulsed renal pedicle or mesenteric vessels), vascular neoplasia (e.g., hemangiosarcoma, other vascular tumors or tumors with necrotic centers), feline hepatic amyloidosis, or coagulopathies (see Chapter 5; Figure 10-3). Physical examination usually reveals abrasions or pain in traumatized animals. Inspection for petechia (including fundic examination), rectal and fecal examination for melena and hematochezia, palpation for hemarthrosis (i.e., swollen, painful joints), and urinalysis looking for hematuria help identify coagulopathies. Without historic or physical findings suggesting trauma, a complete blood count (CBC) and coagulation profile become essential. PCV reveals whether the erythron mass is reduced if there has been time for fluid redistribution and RBC regeneration.

Samples must be obtained before initiating fluid therapy. Schistocytes and acanthocytes (see Chapters 2 and 3) suggest microangiopathic damage because of vascular neoplasia (e.g., hemangiosarcoma) or disseminated intravascular coagulation (DIC). Scanning a blood smear should detect thrombocytopenia severe enough to cause hemorrhage (see Chapter 5). Severe acute hemorrhage

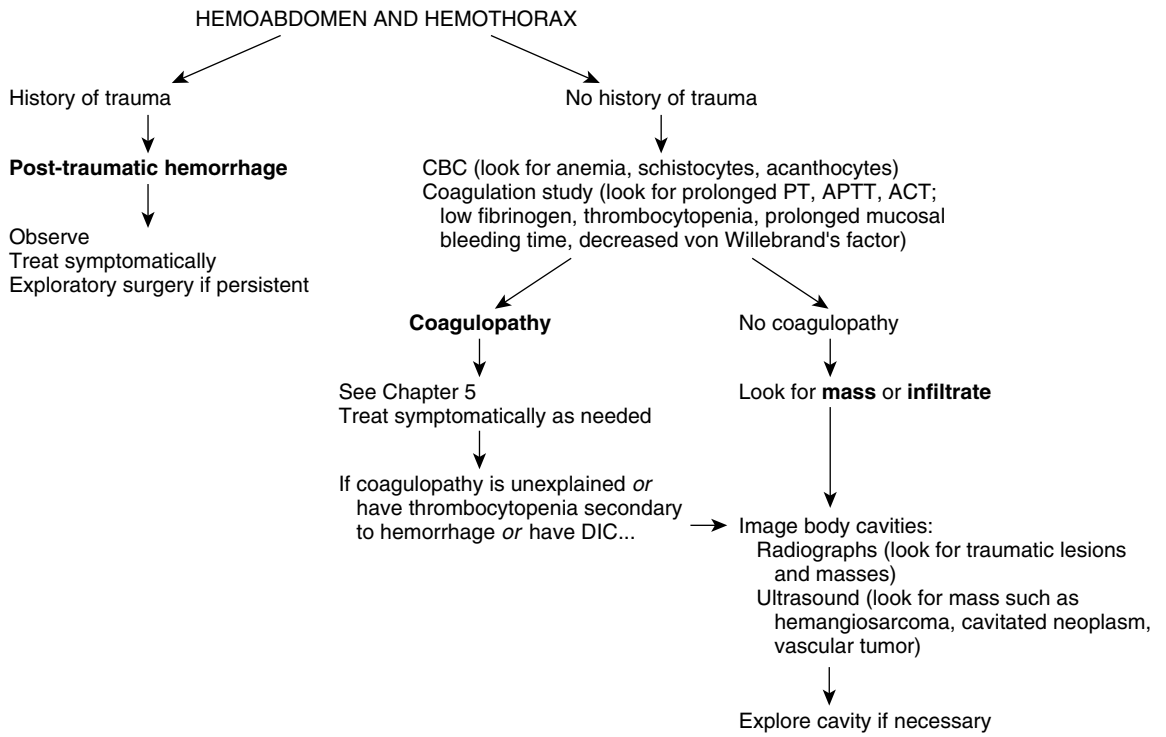

FIGURE 10-3. Diagnostic considerations in animals with hemoabdomen or hemothorax. *ACT*, Activated clotting time; *APTT*, activated partial thromboplastin time; *CBC*, complete blood count; *DIC*, disseminated intravascular coagulation; *FIP*, feline infectious peritonitis; *PIVKA*, proteins induced by vitamin K absence; *PT*, prothrombin time.

not caused by thrombocytopenia initially increases the platelet count. An activated coagulation time (ACT) and buccal mucosal bleeding time (BMBT) (see Chapter 5) detect many hemostatic defects. Thoracic radiographs may reveal pleural fluid, lymphadenopathy (e.g., sternal lymph node), or frank metastasis. Pleural fluid, if present, must be sampled. Ultrasonography may find vascular tumors, usually associated with hepatic or splenic hematomas, and occasionally reveals the site of active bleeding. Animals with persistent or vigorous abdominal hemorrhage may require blood volume replacement, hemoglobin infusion, empiric vitamin K1 treatment, (0.5 to 1.5 mg/kg subcutaneously [SC] or intramuscularly [IM]) and often laparotomy if hemostatic defects have been eliminated.

Bilious effusions caused by gallbladder or common bile duct rupture may derive from blunt abdominal trauma, necrotizing cholecystitis or choledochitis, or cholelithiasis. Biliary tree perforation may be immediate or delayed after blunt or surgical injury (see Table 10-3). Affected animals usually have low-grade abdominal pain, lethargy, fever, jaundice, and abdominal effusion. Pale or acholic feces indicate deviation of bile from the intestines. Serum alkaline phosphatase (SAP), gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), and aspartate transferase (AST) activities are invariably increased. The appearance and severity of jaundice depends on the underlying cause and chronicity of biliary tree rupture. Initially there may be large volumes of fluid with few clinical signs. Exposure of cell membranes to bile acids and lysolecithin promotes the transmural migration of enteric microbes causing peritonitis. Sometimes focal bile peritonitis is contained by the omentum. If a pocket of fluid is found ultrasonographically, it can be aspirated using a spinal needle to confirm the diagnosis. A ruptured gallbladder may not be well visualized ultrasonographically. Diffuse bile peritonitis is usually cytologically obvious: high numbers of neutrophils and macrophages plus free and engulfed bile. The specimen is usually golden-brown or golden-green, and the fluid

is turbid. Higher bilirubin concentration in the effusion than in serum is common, but this determination is usually unnecessary for diagnosis. Fluid samples for accurate diagnosis of bile peritonitis are collected from the immediate area of leakage.

Uroabdomen occurs when urine leaks and pools in the peritoneal cavity. Affected animals sometimes have apparently normal voiding (e.g., a single ruptured ureter; a bladder avulsion when a fibrous tract permits voiding into the urethral lumen). Avulsion of a ureter at the renal pedicle causes retroperitoneal fluid accumulation. Trauma is the major cause, but cystocentesis or neoplasia may be responsible. The degree of azotemia varies; if virtually all urine produced accumulates in the abdomen, rapid onset of azotemia is expected. Most patients develop a vaguely painful abdomen, lethargy, fever, and dehydration. Markedly increased blood urea nitrogen (BUN) and creatinine concentrations, hyperphosphatemia, hyponatremia, hyperkalemia, and metabolic acidosis are expected. The effusion is slightly turbid, blood-tinged, and yellow. Fluid creatinine concentration is markedly higher than peripheral blood creatinine. No substantial difference is found in the fluid and serum urea nitrogen concentrations because of the very small size of the urea molecule and dispersion in body water. An intravenous (IV) urogram (followed by a retrograde urethral cystogram, if necessary) locates the damaged area. Urinary drainage and abdominal lavage rapidly corrects electrolyte and acid-base derangements.

Pancreatitis sometimes causes diffuse peritonitis and copious effusion. Clinical pathologic changes are discussed in Chapter 9; ultrasonography is usually diagnostic in dogs. Effusions are grossly turbid and sometimes have a lipid surface interface after refrigeration and centrifugation. Inflammation is characterized by large numbers of neutrophils and macrophages; the latter often contain many small to large clear or refractile vacuoles (probably engulfed lipid). Pancreatic enzyme activity in the effusion (i.e., lipase) can markedly exceed values measured in peripheral blood.

Ruptured "cystic" lesions in the liver, kidneys, pancreas, or prostate occasionally cause abdominal effusion (see Figure 10-1). Fluid located within large cysts is sometimes misidentified as free abdominal effusion. Cystic fluid is evaluated for underlying malignancy or infection. Polycystic hepatic or renal disease is more common in cats (i.e., Persians, Himalayans). Hepatic and renal cysts may contain serous fluid or, more rarely, fluid contaminated with bile or urine, respectively. Perirenal pseudocysts are more common in cats, especially older males. Although rare, pancreatic cysts may be benign, represent post-pancreatitis abscessation, or be the result of malignancy (i.e., pancreatic adenocarcinoma). Ultrasound-guided cyst aspiration is the least invasive and most cost-effective method of determining if resection or drainage is needed. Paraprostatic or prostatic cysts may become exceedingly large. Examination of cystic fluid is important to eliminate infection or neoplasia. If perirectal cysts are identified and the clinician is unsure of urinary bladder position, the urinary bladder should be decompressed with a catheter; cyst fluid should then be aspirated and analyzed for creatinine.

Pleural Effusions

The general approach to differential diagnosis of pleural effusion is similar to that described for abdominal effusions (Figure 10-4). The pleura is not as readily accessible by exploratory surgery and is less easily visualized by ultrasonography, however. Animals with pleural effusion often have rapid, shallow breathing with accentuated abdominal effort. Radiographs should include right lateral and ventrodorsal views. Radiographically, pleural fluid is characterized as either free (will move upon patient repositioning) or encapsulated (nonmovable with positional changes).

Pure transudates are less common in the pleural space than in the abdomen (Figure 10-5). These effusions signal severe hypoalbuminemia and thoracic vascular hypertension. Pure transudates also occur in animals overhydrated with crystalloid solutions. Overhydration causing pleural and pulmonary fluid retention occurs most commonly with incipient cardiac disease (e.g., asymptomatic cardiomyopathy).

Modified transudates are the most common type of pleural effusion (see Figure 10-5). Obstructive effusions can be serous to serosanguineous, have a specific gravity from 1.015 to 1.040, and total solid concentrations greater than or equal to 2.5 g/dl. Cellularity is usually mixed with RBCs, lymphocytes, and fewer neutrophils, eosinophils, macrophages, and mesothelial cells. These effusions eventually become modified with a more

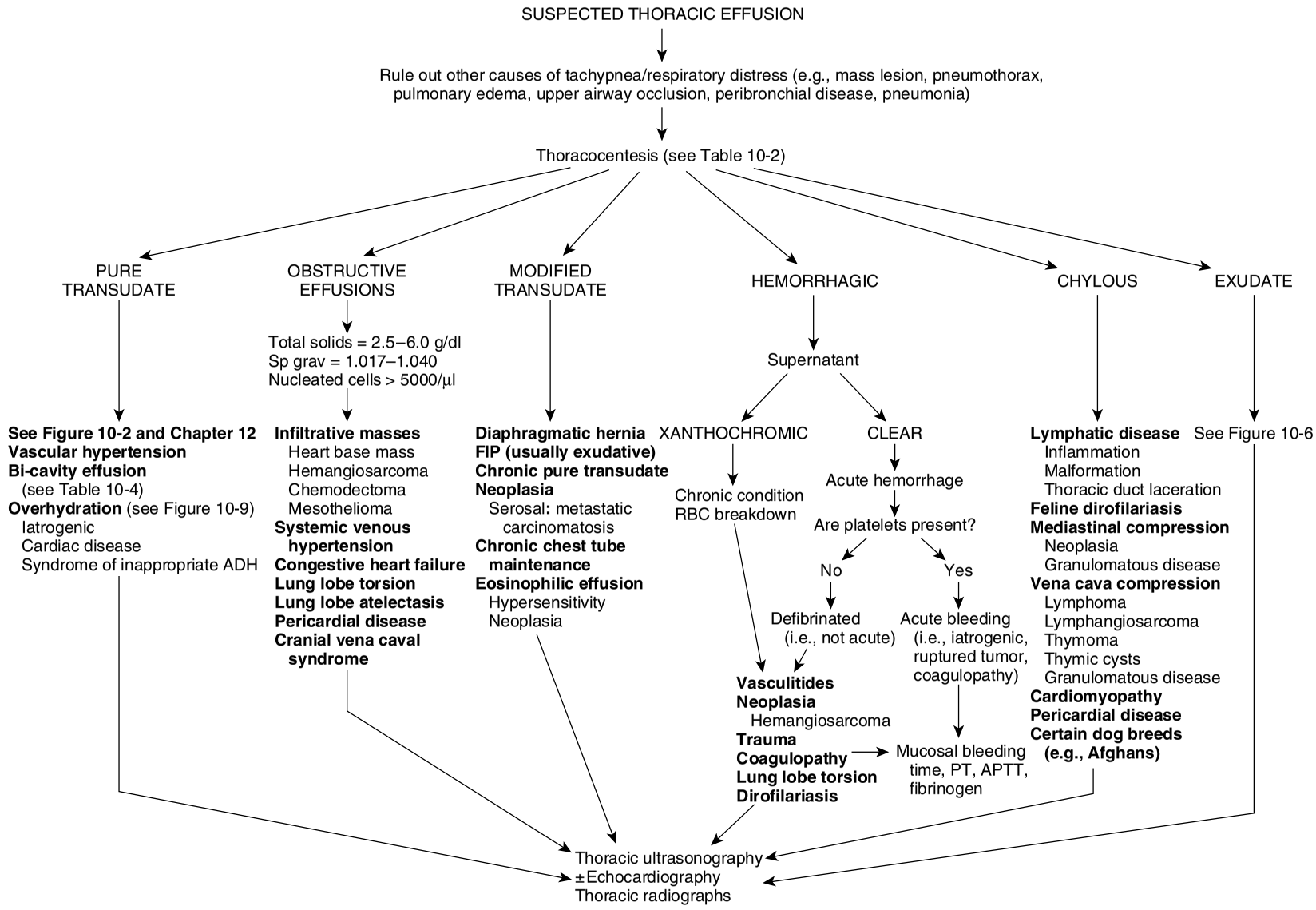

FIGURE 10-4. Diagnostic considerations in animals with suspected thoracic effusion. *ADH*, Antidiuretic hormone; *APTT*, activated partial thromboplastin time; *FIP*, feline infectious peritonitis; *PIVKA*, proteins induced by vitamin K absence; *PT*, prothrombin time; *RBC*, red blood cell.

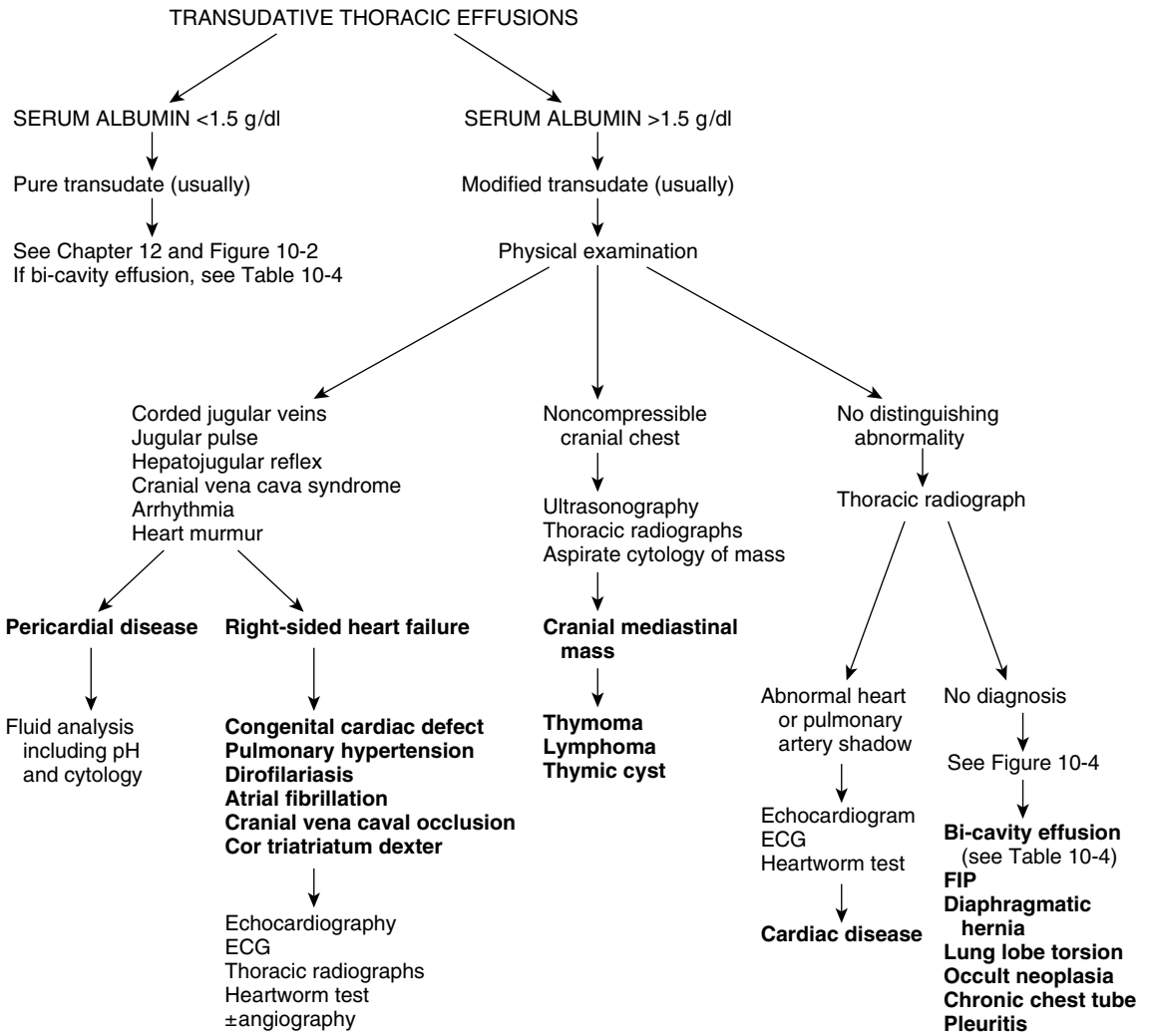

FIGURE 10-5. Diagnostic considerations in animals with pleural transudates. *ECG*, Electrocardiogram; *FIP*, feline infectious peritonitis.

inflammatory appearance. Physical examination may disclose underlying disease (e.g., gallop rhythm, cardiac murmur, loss of normal compression of the anterior chest in cats with an enlarged heat or mediastinal mass). Trauma plus a vague "emptiness" of the abdomen and auscultation of borborygmi in the thorax suggests diaphragmatic hernia; dogs with right-sided cardiac failure usually develop ascites, and cats often form pleural effusion with cardiac failure. Benign or neoplastic pericardial effusion, constrictive or restrictive pericarditis, and right atrial hemangiosarcoma cause pleural effusion associated with pericardial tamponade in dogs. Occasionally, animals with a cranial mediastinal mass and associated pleural effusion have *cranial vena cava syndrome* (i.e., submandibular edema, jugular engorgement and pulses, injection of the conjunctival blood vessels indicating impaired cranial venous return, lymphatic return, or both). Thymomas, thymic cysts, lymphoma, invasive thyroid adenocarcinoma, and jugular thrombosis are the most common causes of this syndrome.

Thoracic radiography should be performed before thoracocentesis to minimize the possibility of iatrogenic lung laceration, to disclose cardiomegaly (suggesting cardiomyopathy or pericardial effusion), and to detect gas entrapped within visceral structures (i.e., diaphragmatic hernia) or abscess.

Removing the effusion followed by radiography may permit identification of masses and consolidated lung lobes. Thoracic ultrasonography is enhanced by voluminous thoracic effusion; ultrasound-guided aspiration of masses may provide a definitive diagnosis. Most animals require sedation before needle-targeted thoracic sampling of lesions deeper than the peripheral pleural space.

Thoracic exudates (Figure 10-6; see Figure 10-4) necessitate cytologic examination for infectious organisms. *Pasteurella multocida* is the most common reported aerobic isolate. *Actinomyces* and *Nocardia* are often found in dogs. Multiple bacterial species are common in feline pyothorax. Septic exudates typically are turbid, cream-colored, or seropurulent and contain degenerate WBCs with or without multiple bacterial forms. Exudates associated with pure *Actinomyces* infection may have minimal or no WBC degeneration; dual infections with *Nocardia* are common, and mixed populations of organisms may be found in thick or thin red-brown exudates containing degenerate WBCs and "sulfur granules." Including colored flecks from the exudate on smears improves cytologic visualization of actinomycosis-nocardiosis. These organisms are presumptively diagnosed when beaded, branching filaments (Color Plates 4B and 4C) are found. Most *Nocardia* spp. stain acid fast with a modified acid-fast stain; *Actinomyces* spp. do not. Both organisms produce exudates containing sulfur granules, but only *Actinomyces* produces these within tissue. Thoracic radiography in animals with pyothorax may disclose pulmonary parenchymal involvement. Effusions persist as long as a chest tube is retained. Sequential evaluation of cytologic specimens can be used to estimate patient response to treatment and guide the propriety of tube removal.

Nonseptic exudates with a hemorrhagic component are associated with lung lobe torsion (suggested by radiographic evidence of malpositioned main stem bronchi, persistence of entrapped air within bronchi of twisted lobes, and prolific fluid production). Bronchoscopy may reveal a twisted-off bronchus. Ultrasonography may assist by evaluating lobe perfusion. Nonseptic exudates also are associated with idiopathic pleuritis, infectious pneumonia (e.g., mycoplasmic pneumonia), and various tumors. FIP can cause pyogranulomatous pleural effusion that is light yellow, viscous, and has a high protein concentration. A background of proteinaceous material is commonly observed on fluid cytology.

Thoracic neoplasia often induces pleural effusion; the most common is mediastinal lymphoma. In dogs, lymphocytes are usually the predominate cell; these may lack overt malignant characteristics. Aspiration of mediastinal masses or lymph nodes or other more accessible enlarged nodes may be diagnostic. In cats, exfoliated lymphoblasts are common. It is important to differentiate thymomas from lymphoma, because the former may have a better prognosis; this may require tissue sampling. Malignant or benign thymic cysts also may cause pleural effusion. Mesotheliomas pose a great diagnostic challenge; definitive diagnosis requires tissue biopsy.

Hemorrhage is usually caused by trauma. Radiographs often reveal rib fractures, pulmonary consolidation, lung lobe torsion, or pneumothorax. Nontraumatic hemorrhagic pleural effusions are usually the result of bleeding neoplasia; however, coagulopathy also must be considered (e.g., minor trauma can cause substantial bleeding in dogs with severe von Willebrand's disease or vitamin-K depletion from warfarin-like rodenticides; see Chapter 5). Other nontraumatic causes of hemothorax include lung lobe torsion, pulmonary abscessation, pulmonary infarction, dirofilariasis, and (rarely) *Spirocerca lupi*–associated aortic aneurysm. Disseminated pulmonary hemangiosarcoma causing hemorrhagic effusion is difficult to diagnose antemortem in dogs. Pulmonary aspirates or open-chest biopsy carry a high mortality rate because of tension pneumothorax and worsening hemorrhage.

Chylous effusions are more common in the thorax than in the abdomen. They may be idiopathic or associated with underlying disease. Associated disorders are summarized in Figure 10-4. Some breeds (e.g., Afghan hounds) may have a congenital propensity. Thoracic ultrasonography or postdrainage radiography can assist in identifying underlying conditions. Contrast lymphangiography performed by cannulating mesenteric lymphatics or the thoracic duct sometimes elucidates the site of chyle leakage.

Bicavity Effusions

The list of differentials includes several diseases (Table 10-4). Tumors and cardiac disease are particularly common causes.

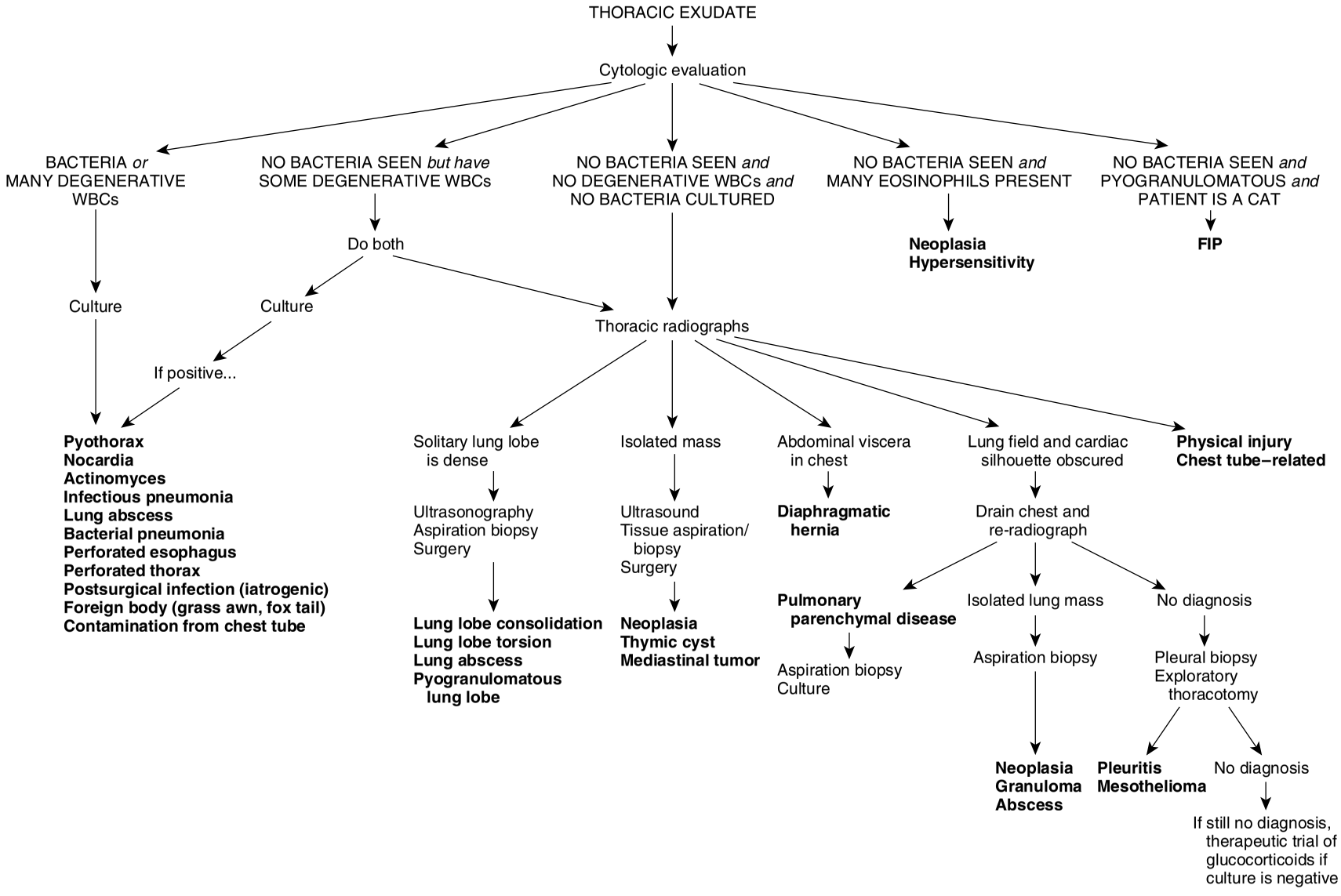

FIGURE 10-6. Diagnostic considerations in animals with thoracic exudates. *FIP*, Feline infectious peritonitis; *WBCs*, white blood cells.

TABLE 10-4. Conditions Associated with Bicavity Effusions

Cardiovascular conditions
Idiopathic hemorrhagic pericardial effusion
Constrictive pericardial disease
Biventricular cardiac failure
Congestive cardiomyopathy
Hypertrophic cardiomyopathy
Idiopathic pulmonary hypertension
Right ventricular thromboembolism
Caudal vena cava thromboembolism
Congenital obstruction: caudal vena cava
Pancreatitis
Bile peritonitis
End-stage hepatic disease
Feline infectious peritonitis
Neoplastic conditions
Right atrial fibroma
Metastatic adenocarcinoma
Lymphoma
Hemangiosarcoma
Mesothelioma
Cholangiocellular carcinoma
Chemodectoma
Prostatic adenocarcinoma
Diffuse carcinomatosis

Pericardial Effusions

Pericardial effusion is usually associated with pericardial irritation and inflammation, hemorrhage, or central venous congestion. Animals with congestive cardiomyopathy may have small-to-moderate amounts of pericardial fluid. Acutely disabled patients require emergency pericardiocentesis, but it should not be performed indiscriminately.

Pure transudates are caused by severe hypoalbuminemia. *Modified transudates* are found with right-sided heart failure, pericardial cysts, and uremic pericarditis. *Exudates* are found with FIP, bacterial pericarditis, and coccidioidomycosis. Septic pericarditis is rare. Chylopericardium is rare but may develop consequent to mediastinal venous hypertension (e.g., cardiomyopathy). Trauma, neoplasia, or coagulopathy can cause hemopericardium. Most dogs with pericardial tamponade have modified transudates or exudates (e.g., benign pericardial effusion in approximately 50% of cases; neoplasia including hemangiosarcoma of the right atrium [most common], chemodectoma, metastatic adenocarcinoma, mesothelioma). Cytologic differentiation of benign from malignant pericardial effusion is difficult. Fluid pH (determined using a urine dipstick) may help: Effusions with a pH greater than or equal to 7.0 are usually malignant, whereas a pH less than or equal to 6.5 suggests benign effusions (Jackson, Richter, and Launer, 1999). However, this feature appears variable. Contrast radiographs, ultrasonography, thoracoscopy, or exploratory surgery are often needed to differentiate benign from malignant disease. Pericardial biopsy via thoracoscopic sampling is the least invasive reliable method of tissue retrieval. Most cats with pericardial disease have cardiac disease (i.e., cardiomyopathy, valve abnormalities), neoplasia (i.e., lymphoma, metastatic carcinomas), chronic renal disease (uremic pericarditis), coagulopathies or more rarely, bacterial infection. Intrapericardial cysts rarely cause serosanguineous modified transudates or exudates in dogs; diagnosis is based on echocardiography.

Joint Effusions

Joint fluid should be analyzed in patients with swollen, fluctuant, or painful joints not caused by degenerative joint disease. Joint pain may be subtle; joints should be carefully palpated for mobility, swelling, and discomfort. Synovial fluid analysis distinguishes noninflammatory from inflammatory conditions; examination of joint fluid may be helpful in animals with fever of unknown origin. Many immune-mediated diseases involve joints. Very small amounts of fluid are available from most normal joints; therefore, collecting relatively large volumes suggests effusion. Acute and inflammatory conditions cause effusion, whereas chronic and noninflammatory disorders produce joint enlargement due to soft tissue swelling but yield little fluid. If arthrocentesis is performed to evaluate fever or unexplained lameness in an animal without swollen joints, at least two or three joints should be sampled (carpal and tarsal usually preferred).

High cell counts cause turbidity. Homogeneously bloody fluid indicates hemorrhage, and fluid containing "streaks" of blood indicates iatrogenic bleeding. Culture, nucleated cell counts, differential cell counts, and estimation of fluid viscosity (mucin tests) are desirable (Table 10-5, Figure 10-7). Normal synovial fluid has a "sticky" viscosity forming a viscous strand when dripped from a needle. Viscosity is reduced in most types of joint effusion. If small amounts of fluid are obtained, however, smears to determine relative cellularity and the predominant cell type are the first priority. Cytology usually discloses if inflammation is present. Inflammation is indicated by finding greater

TABLE 10-5. Analysis of Joint Effusions

	NONINFLAMMATORY JOINT DISEASE			INFLAMMATORY JOINT DISEASE		
	DEGENERATIVE JOINT DISEASE	NEOPLASTIC JOINT INVOLVEMENT	HEMARTHROSIS	INFECTIOUS INFLAMMATION	NONINFECTIOUS INFLAMMATION	NORMAL
Color	Light yellow	Light yellow—blood-tinged	Bloody, xantho-chromic	Variable: yellow, blood-tinged, bloody	Variable: yellow, blood-tinged	Straw-colored
Turbidity	Clear—slightly turbid	Mild to moderate turbidity	Turbid	Turbid to purulent	Variable: slight to moderate turbidity	Clear
Viscosity	Normal	Normal to reduced	Reduced	Reduced	Reduced	Viscous
Mucin Clot Test	Normal firm	Normal firm	Normal to slightly friable	Friable	Friable	Firm
CYTOLOGY:						
RBC	Few	Few to many	Many Erythrophago-cytosis	Moderate	Few to moderate	Rare
WBC/μl	<3000	Variable	Variable	40,000-250,000	Many but variable	0-2900
Neutrophils	Few (<20%)	Moderate	Moderate	Many (usually >90%)	Many but variable	0-10%
Degenerative changes	Absent	Absent	Absent	May be present	None to mild	None
Lymphocytes	Few to moderate	Few to moderate	Rare	Few	Few to moderate	Few
Synoviocytes	Common	Few	Rare	Few to moderate	Few to moderate	Few
Macrophages	Few to moderate	Few to moderate	Moderate if chronic	Few to moderate	Few to moderate	Rare
Microorgan-isms	None	None	None	May be present	None	None
Neoplastic cells	None	Variable	None	None	None	None
Others					May see LE cells	

LE = lupus erythematosus; RBC = red blood cell; WBC = white blood cell.

than 3000 WBC/μl or when greater than 15% of the cells are neutrophils.

Sepsis is suggested by degenerative neutrophils and is confirmed by seeing or culturing bacteria. Unfortunately, many dogs with septic joints have minimal to no degenerative neutrophils. Concurrent antibiotic therapy may cause underestimation of infection because of suppression of degenerative neutrophil changes, suppressed numbers of organisms, and negative culture results. Some agents may not cause degenerative neutrophils and are not cytologically detectable (e.g., mycoplasma, rickettsia, L-form bacteria, viruses [calicivirus in cats, postvaccinal arthritides]). Most canine polyarthritis is nonspecifically immune-mediated. Feline polyarthritis (i.e., chronic progressive polyarthritis) is linked (statistically) with feline syncytium-forming virus (FeSFV) and feline leukemia virus (FeLV) infection. If minimal amounts of fluid are aspirated, the syringe and needle can be rinsed with broth-enrichment media and this rinse cultured; synovial fluid preincubated in blood culture media for 24 hours also may yield more positive cultures. In chronic low-grade infections, culturing synovial tissue biopsies may be more productive than synovial fluid culture. If synovial fluid infection is present, blood and urine cultures plus echocardiography are reasonable next steps. Omphalophlebitis may be the source of infection in young animals. Serologic testing for borreliosis (i.e., Lyme disease) may help diagnose inflammatory arthritis of unknown cause; however, interpretation of

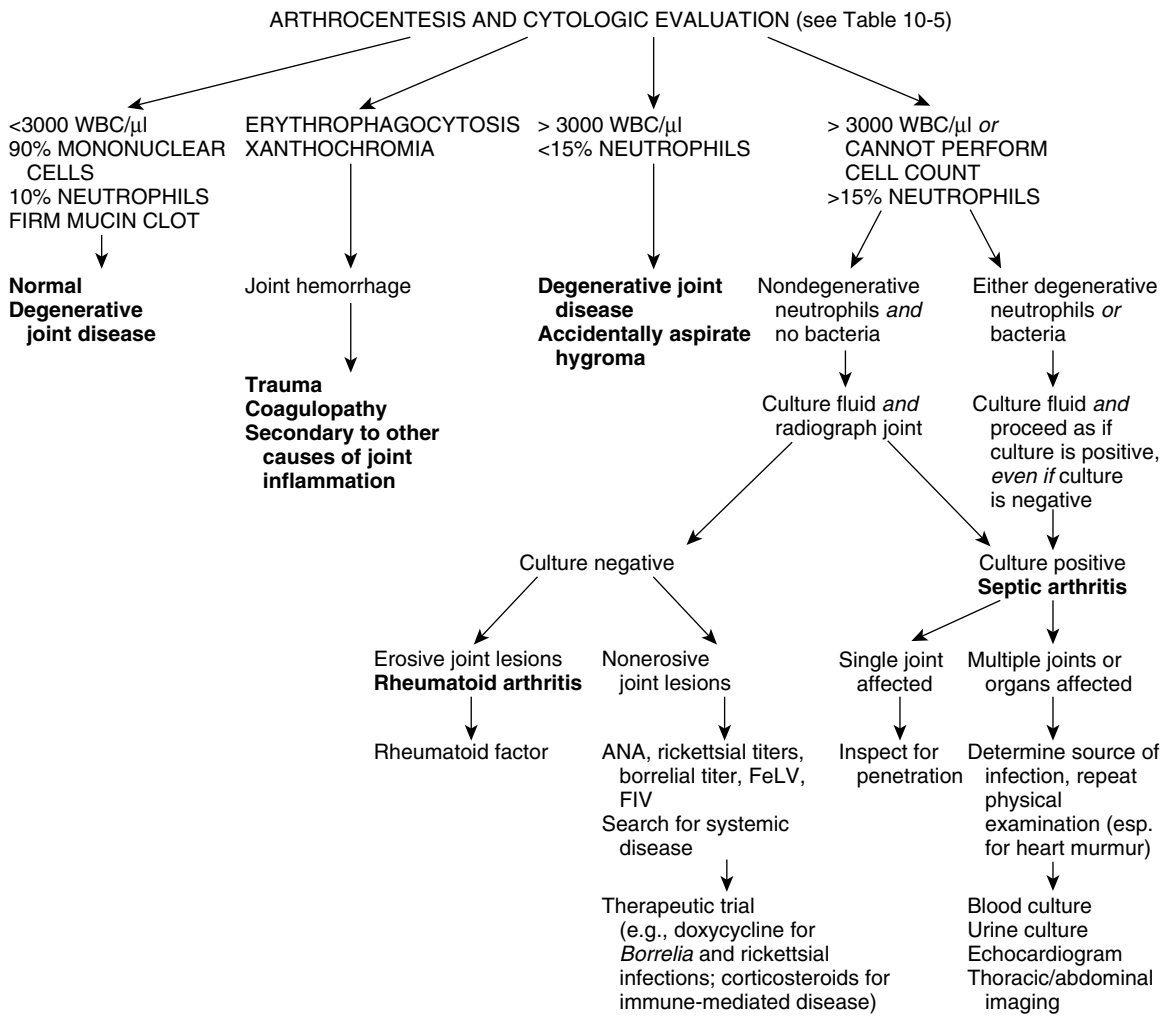

FIGURE 10-7. Diagnostic considerations in animals with joint effusion. *ANA,* Antinuclear antibodies; *FeLV,* feline leukemia virus; *FIV,* feline immunodeficiency virus; *WBC,* white blood cell.

Borrelia titers is difficult (see Chapter 15). Response to therapy may be the most compelling basis for diagnosing Lyme disease or rickettsial polyarthropathies.

Exudative joint disease without evidence of sepsis is categorized as erosive or nonerosive based on radiographs. Rheumatoid arthritis is classically erosive (see Figure 10-7). Nonerosive arthritides are more difficult to definitively diagnose and categorize. Systemic lupus erythematosus (SLE) and arthritis associated with a variety of underlying diseases are more common. Ragocytes, lupus erythematosus (LE) cells, or both indicate immune-mediated disease (see Color Plate 5A). Submission of sera for antinuclear antibody (AN) and rheumatoid factor titers (see Chapter 12), blood for LE cell preparations, and synovial biopsies for histologic evaluation may implicate immunodestructive processes. Chronic, progressive feline polyarthritis usually occurs in males and has two forms. The periosteal-proliferative form is most prevalent, primarily affecting young adult cats and causing tenosynovitis followed by nondeforming periarticular periosteal proliferation and subchondral bone erosions. The deforming or rheumatoid-like arthritis form has an insidious onset in older cats and is associated with severe subchondral bone destruction, joint instability, and deformity. Feet, carpi, and tarsi are severely and symmetrically affected.

The most common cause of noninflammatory joint disease is degenerative arthritis because of trauma or joint instability. Other causes include hemarthrosis, neoplasia, genetic or developmental disorders, dietary or nutritional deficiencies or excesses, and miscellaneous causes (e.g., hypertrophic osteopathy).

Edema

Edema signals underlying disease. Hypoproteinemia causes edema fluid with total solids less than 1 g/dl, whereas venous congestion, lymphedema, or inflammation generate fluid with total solids greater than 2.5 g/dl. In the latter case, total and differential cell counts may distinguish inflammatory (e.g., vasculitis) from noninflammatory causes.

Regional edema is usually caused by inflammation or vascular or lymphatic obstruction (Figure 10-8). Lymphadenopathy is an indication for lymph node aspirates for cytology and culture. Congenital or acquired arteriovenous fistulae are rare but may cause localized edema. These may be detected by palpable fremitus or bruit, ultrasonography, or angiography. Acquired lymphatic insufficiency after trauma, surgery, or regional infections commonly causes regional edema. Lymphangiograms are usually not indicated in acute disorders. A fine-needle aspiration of involved tissues or regional lymph nodes and aspiration of edema fluid may be helpful. Lymphatic cording is occasionally palpated in animals with lymphatic obstruction or inflammation. Congenital malformation or degenerative lymphatic disorders may cause lymphedema in young animals; lymph nodes are sometimes atrophied or absent. Limb edema and tail edema are most commonly recognized. Affected animals develop overtly swollen appendages (single or multiple) but lack significant physical disability.

Generalized edema or *anasarca* is usually dependent, especially occurring in distal extremities (Figure 10-9). Overhydration, right-sided congestive heart failure, and hypoalbuminemia are the most common causes (Table 10-6). Iatrogenic overhydration does not typically induce marked generalized edema unless another underlying factor (e.g., hypoalbuminemia, anuria-oliguria) exists. Iatrogenic, generalized edema imparts an obvious change in skin turgor (e.g., jelly-like consistency) that usually resolves within 48 hours of discontinuing fluids. Animals with normal serum albumin concentrations that develop edema because of overhydration may have insidious cardiac disease, high-output cardiac failure (associated with severe anemia), or anuric-oliguric renal failure.

Serum albumin must be less than or equal to 1.5 g/dl to cause anasarca. This generates a pure transudate in body cavities. Patients with generalized edema from vasculitis usually have perivascular hemorrhage (i.e., petechial hemorrhages, microhematuria) or microangiopathic effects (i.e., schistocytes). Total body water retention because of inappropriate elaboration of antidiuretic hormone (ADH) (see Chapter 6) coexists with other disorders that overshadow its presence. Retention of sodium because of increased renin, angiotensin, and aldosterone occurs in patients with severe renal, hepatic, or cardiac disease having hypoalbuminemia. Systemic hypertension often occurs when the renin-angiotensin axis is the primary abnormality. History, physical examination, serum albumin determination, thoracic radiographs, cardiac evaluations (i.e., ECG, echocardiography), CVP determinations, and urinalysis assist in confirming the cause of anasarca (see Figure 10-9). Finding a jugular pulse or abnormal hepatojugular reflex suggests intrathoracic disease. If an underlying cause is not found, physical examination and laboratory data are reviewed looking for evidence of vasculitis (i.e., petechiation, fundic examination for microvascular lesions, microscopic hematuria, blood smear looking for schistocytes). Skin biopsies performed in areas of bruising or cutaneous lesions should be taken to investigate vasculitis. Collecting edema fluid and analyzing its protein content helps differentiate inflammatory causes from hypoalbuminemia.

Scrotal Effusions

Scrotal effusion usually develops when abdominal fluid enters the scrotum via the inguinal rings. Severe orchitis or testicular torsion may also be responsible and can often be discerned by ultrasonography and aspiration cytology. Because scrotal edema may be caused by vasculitis (e.g., Rocky Mountain spotted fever), evaluation for systemic infection is important. If effusion is localized to the scrotum, castration may be diagnostic and therapeutic.

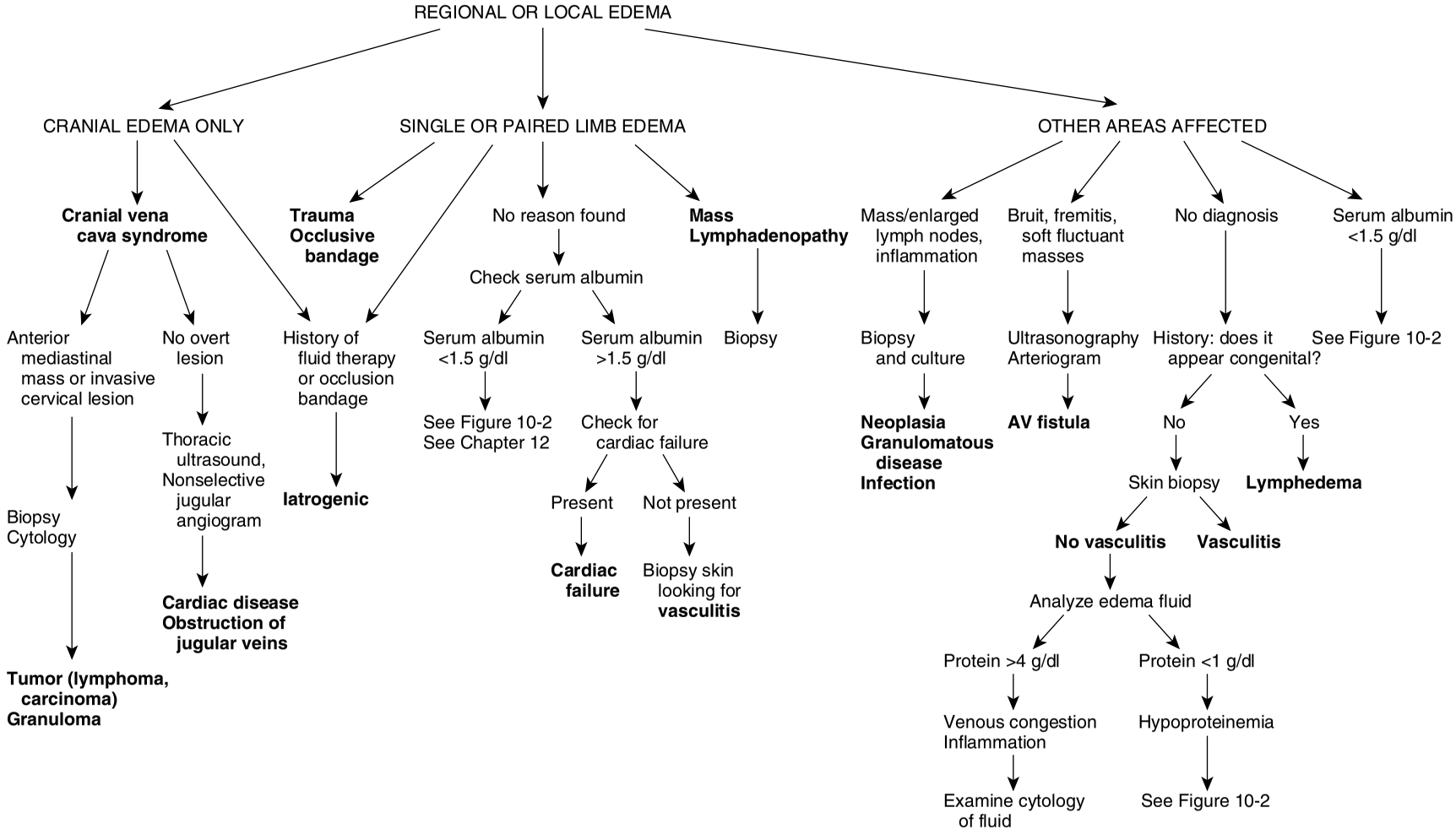

FIGURE 10-8. Diagnostic considerations in animals with regional or localized edema. *AV,* Arteriovenous.

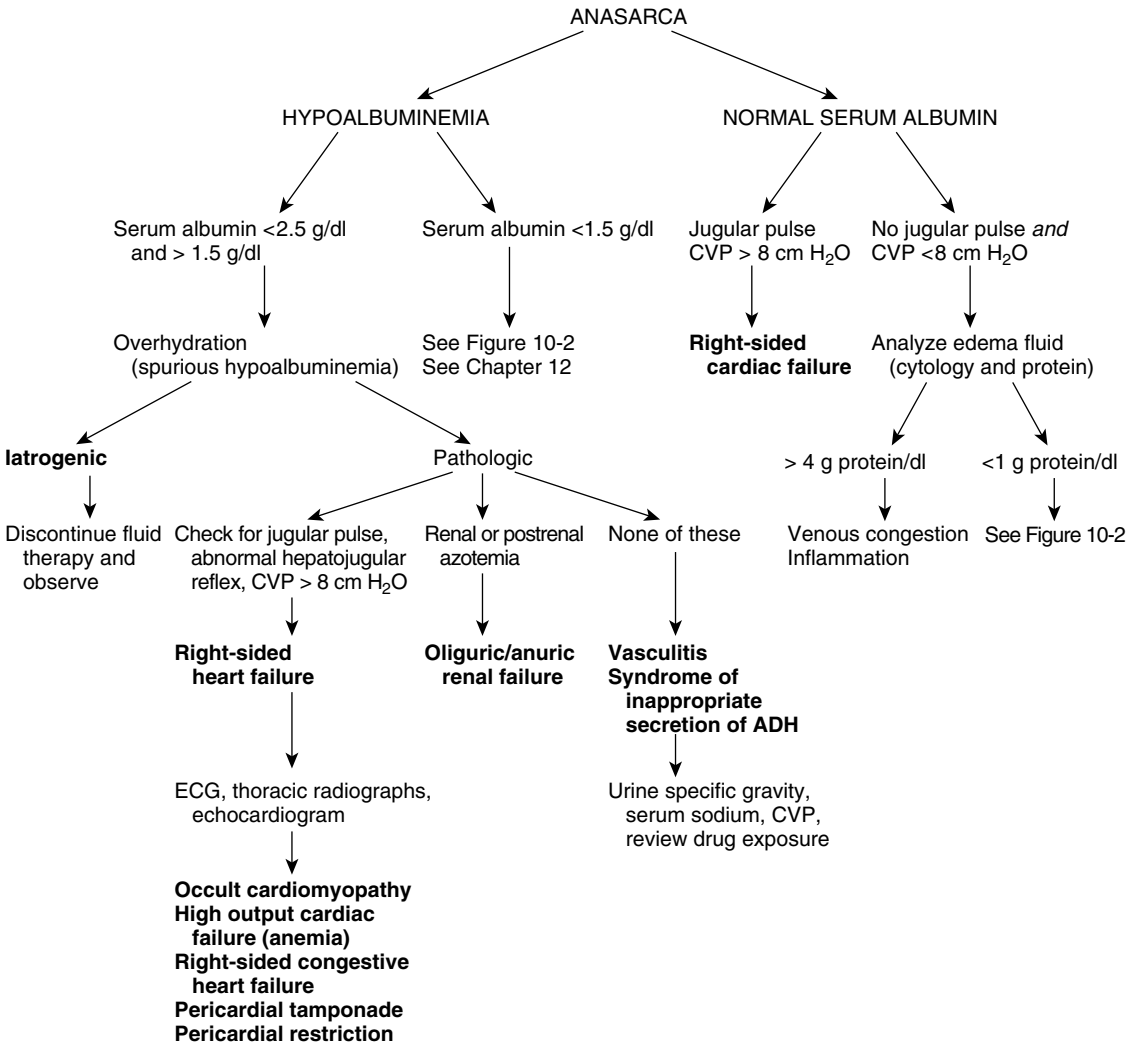

FIGURE 10-9. Diagnostic considerations in animals with anasarca. *ADH*, Antidiuretic hormone; *CVP*, central venous pressure; *ECG*, electrocardiogram.

TABLE 10-6. Conditions Associated with Anasarca

Iatrogenic overhydration
Calculated or administered wrong fluid volumes
Acute renal failure
Anuria with fluid administration
Hepatic failure
Hypoalbuminemia
Portal hypertension
Increased body sodium and water retention
Nephrotic syndrome
Hypoalbuminemia
Increased body sodium and water retention
Vasculitis
Increased vascular permeability
Immune-mediated (e.g., systemic lupus erythematosus)
Infectious diseases (e.g., rickettsial infections)
Hypersensitivity reaction

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11

Ned F. Kuehn and Grant H. Turnwald

Respiratory Disorders

RESPIRATORY PROBLEMS

Dyspnea

Dyspnea (also called *respiratory distress*) is an inappropriate degree of breathing effort based on respiratory rate, rhythm, and character. Dyspnea is a common sign associated with a wide variety of respiratory and nonrespiratory disorders (Kuehn and Roudebush, 1991a). Physical examination is the first step in diagnosis. Patients with obstructive respiratory diseases have a breathing pattern characterized by increased depth, rate, and effort. Dynamic obstruction (e.g., laryngeal paralysis) cranial to the thoracic inlet (i.e., the upper airway) causes increased inspiratory effort. Dynamic obstruction (e.g., collapsing trachea) caudal to the thoracic inlet (i.e., lower airway) causes increased expiratory effort. Fixed obstructions (e.g., tracheal tumor) often are associated with increased inspiratory and expiratory effort. Nasal cavity obstruction only causes an obstructive breathing pattern if the animal does not breathe through the mouth. Auscultation of wheezes suggests obstruction, but wheezes are not always found in obstructed patients. Monophonic wheezes (i.e., single tone) may be noted with an elongated soft palate. Alternatively, wheezes can have multiple tones (i.e., polyphonic wheezes), as heard in asthmatic cats.

Dyspnea due to extrarespiratory causes (e.g., severe anemia, severe metabolic acidosis) can have an *apparent* obstructive breathing pattern with an increased intensity of normal lung sounds (i.e., bronchovesicular sounds) but without wheezes.

Diseases limiting ability of the lungs to expand cause a restrictive breathing pattern characterized by an increased rate but a normal to decreased depth with or without increased inspiratory effort. Auscultation may reveal crackles, particularly inspiratory, or a complete absence of lung sounds with certain restrictive lung diseases (e.g., pneumothorax, hydrothorax). It may be difficult to auscultate pulmonary parenchymal abnormalities, especially in small patients with low tidal volumes. Thoracic radiographs of these patients can reveal substantial pulmonary parenchymal disease despite apparently normal lung sounds. Distinction between obstructive and restrictive breathing patterns is not always clear; the pattern of breathing exhibited depends on the relative amount of pathologic change in affected tissues. For example, a dog with severe pulmonary edema may have obstructive (airway fluid) and restrictive (interstitial fluid) disease.

Patients with disorders such as flail chest occasionally have paradoxical movements (i.e., a section of the chest wall collapsing during inspiration and expanding during expiration). Pulmonary vascular diseases can be associated with either inspiratory or mixed inspiratory and expiratory dyspnea.

Common causes of dyspnea are listed in Table 11-1.

Most Useful Tests

After the site of an abnormal breathing pattern has been localized by physical examination, tracheal and thoracic radiographs are typically the most useful next diagnostic step (Figure 11-1). NOTE: Although a collapsed trachea can be diagnosed radiographically, it cannot be ruled out by such, even if inspiratory and expiratory films are made. Fluoroscopy or tracheobronchoscopy may be needed to establish the diagnosis. Transtracheal aspiration (TTA), bronchoalveolar lavage (BAL), or transthoracic fine-needle aspiration (see subsequent headings) may follow if radiography suggests the need for cytologic analysis or culture of the lower airway, especially if coughing is present (Rebar, Hawkins, and DeNicola, 1992). Pharyngoscopy and laryngoscopy are useful in upper-airway obstruction. Tracheobronchoscopy is useful in tracheal and bronchial disorders, especially for diagnosing obstructive disease such as collapsed trachea, and it is mandatory in most pharyngeal and laryngeal abnormalities (see Figure 11-1).

TABLE 11-1. Causes of Dyspnea (Respiratory Distress)

Respiratory Disorders

- Obstructive disease
  - Extrathoracic disease
    - Nasal (only if patient does not breathe through mouth)
    - Stenotic nares
    - Neoplasia
    - Fungal granuloma
    - Foreign body
    - Trauma
    - Epistaxis (blood clots)
  - Nasopharynx
    - Foreign body
    - Neoplasia
  - Pharynx
    - Elongated/edematous soft palate
    - Pharyngeal edema
    - Foreign body
    - Neoplasia
    - Pharyngeal polyps (cats)
  - Larynx
    - Laryngeal paralysis
    - Laryngeal edema
    - Laryngeal collapse
    - Laryngospasm
    - Everted laryngeal saccules
    - Foreign body
    - Neoplasia
  - Trachea
    - Cervical tracheal collapse
    - Foreign body
    - Neoplasia
    - Stenosis
    - Extraluminal compression
    - Traumatic rupture
- Intrathoracic disease
  - Trachea
    - Thoracic tracheal collapse
    - Foreign body
    - Neoplasia
    - Stenosis
    - Extraluminal compression (tumor or granuloma)
    - Parasitic (*Oslerus osleri*)
  - Principal bronchi
    - Bronchial collapse
    - Foreign body
    - Neoplasia
    - Stenosis
    - Extraluminal compression (tumor, granuloma, hilar lymphadenopathy, left atrial enlargement)
    - Parasitic (*Oslerus osleri*)
  - Lower airways and pulmonary parenchyma
    - Bronchial disease
      - Chronic bronchitis (dogs)
      - Bronchial asthma (cats)
    - Pulmonary edema
    - Pneumonia (viral, bacterial, fungal)
    - Aspiration pneumonia
    - Parasitic pneumonitis
    - Hypersensitivity (allergic) lung disease
      - Pulmonary infiltrates with eosinophilia (PIE)
    - Pulmonary hemorrhage
    - Neoplasia
- Restrictive disease
  - Pulmonary disorders
    - Pulmonary fibrosis

Continued

TABLE 11-1. Causes of Dyspnea (Respiratory Distress)—cont'd

- Pulmonary edema
- Interstitial pneumonia
- Pulmonary infiltrates with eosinophilia
- Pleural space or body wall disorders
  - Pneumothorax
  - Pleural effusion
  - Hernia
    - Pleuroperitoneal
    - Pericardioperitoneal
  - Cranial displacement of diaphragm
    - Abdominal mass or masses
    - Abdominal effusion
    - Gastric dilatation
  - Trauma
    - Rib fracture or fractures
    - Flail chest
  - Neoplasia
    - Mediastinum
    - Thoracic wall
  - Obesity

Nonrespiratory Disorders

- Vascular disease
  - Dirofilariasis
  - Pulmonary embolism
- Cardiac disease
- Hematologic
  - Anemia (severe; not true dyspnea)
  - Methemoglobinemia
- Metabolic
  - Acidosis (not true dyspnea)
  - Shock
  - Heat stroke
- Neurologic and neuromuscular disease
  - Central nervous system (CNS)
    - Head trauma
    - Central nervous system inflammatory disease
    - Neoplasia
  - Neuromuscular diseases involving muscles of respiration
  - Myasthenia gravis
  - Polymyopathy
  - Neuropathy
  - Polyradiculoneuropathy

Pleural effusion is always an indication for fluid analysis (see Chapter 10). If dirofilariasis is suspected, a Knott's test, filter test, or *Dirofilaria immitis* antigen (or antibody in cats) test is indicated (see Chapter 15). Although they rarely provide a diagnosis, fecal flotation and Baermann's fecal analysis are inexpensive, noninvasive, and can definitively diagnose respiratory parasites. Serologic testing for systemic mycoses (except histoplasmosis) may be useful in dogs (see Chapter 15).

A complete blood count (CBC) is useful but rarely diagnostic. Allergic or parasitic disease occasionally causes eosinophilia in dogs but is inconsistent in cats (Moise et al, 1989). Dyspnea as the result of severe anemia may also be found. Serum chemistry profile and arterial blood gas analysis are less cost-effective in most of these patients unless the abnormal breathing pattern is nonrespiratory in origin (e.g., severe metabolic acidosis). Percutaneous fine-needle pulmonary aspiration biopsy is occasionally diagnostic in infiltrative disease (e.g., blastomycosis, histoplasmosis, carcinoma), especially if BAL and aspiration of more superficial structures (e.g., lymph nodes) are nondiagnostic. Guiding the needle with radiography, ultrasonography, or fluoroscopy may increase the chance of diagnosis with aspiration of mass lesions. Fine-needle aspiration carries some risk of pneumothorax or hemothorax, however.

Coughing, Including Hemoptysis

Common causes of coughing in dogs and cats are listed in Table 11-2. History is used first to eliminate self-limiting, contagious, infectious diseases. The owner should be carefully questioned to differentiate cough from gagging, because many owners will report gagging as cough. Gagging (not that after a coughing stint) would suggest nasopharyngeal or nonrespiratory disorders (i.e., oropharyngeal, esophageal, or gastrointestinal disease). Differentiation of a productive cough (e.g., bronchopneumonia) from a nonproductive cough (e.g., viral tracheobronchitis) and identification of abnormal breathing patterns (see Table 11-1 and Figure 11-1) are also helpful. Tracheal and thoracic radiographs are essential in patients with chronic cough, hemoptysis, or cardiovascular disease but are less useful in acute infectious tracheobronchial diseases unless secondary pneumonia is suspected. Radiographs can be used to diagnose but not reliably rule out a collapsed trachea. TTA or BAL (especially if combined with bronchoscopy) is often diagnostic of allergic disease, parasitic infections, or bacterial infections and supports a clinical diagnosis of certain chronic bronchial diseases (e.g., chronic bronchitis; Kuehn and Roudebush, 1991b). Bronchoscopy is often needed to diagnose foreign body or obstructive disease. A CBC is seldom helpful unless marked eosinophilia (e.g., allergic or parasitic disease) is present. Fecal flotation and Baermann's fecal analysis are usually less revealing but are indicated because they are easy and cost-effective. Arterial blood gas analysis is rarely diagnostic or cost-effective in coughing patients without dyspnea.

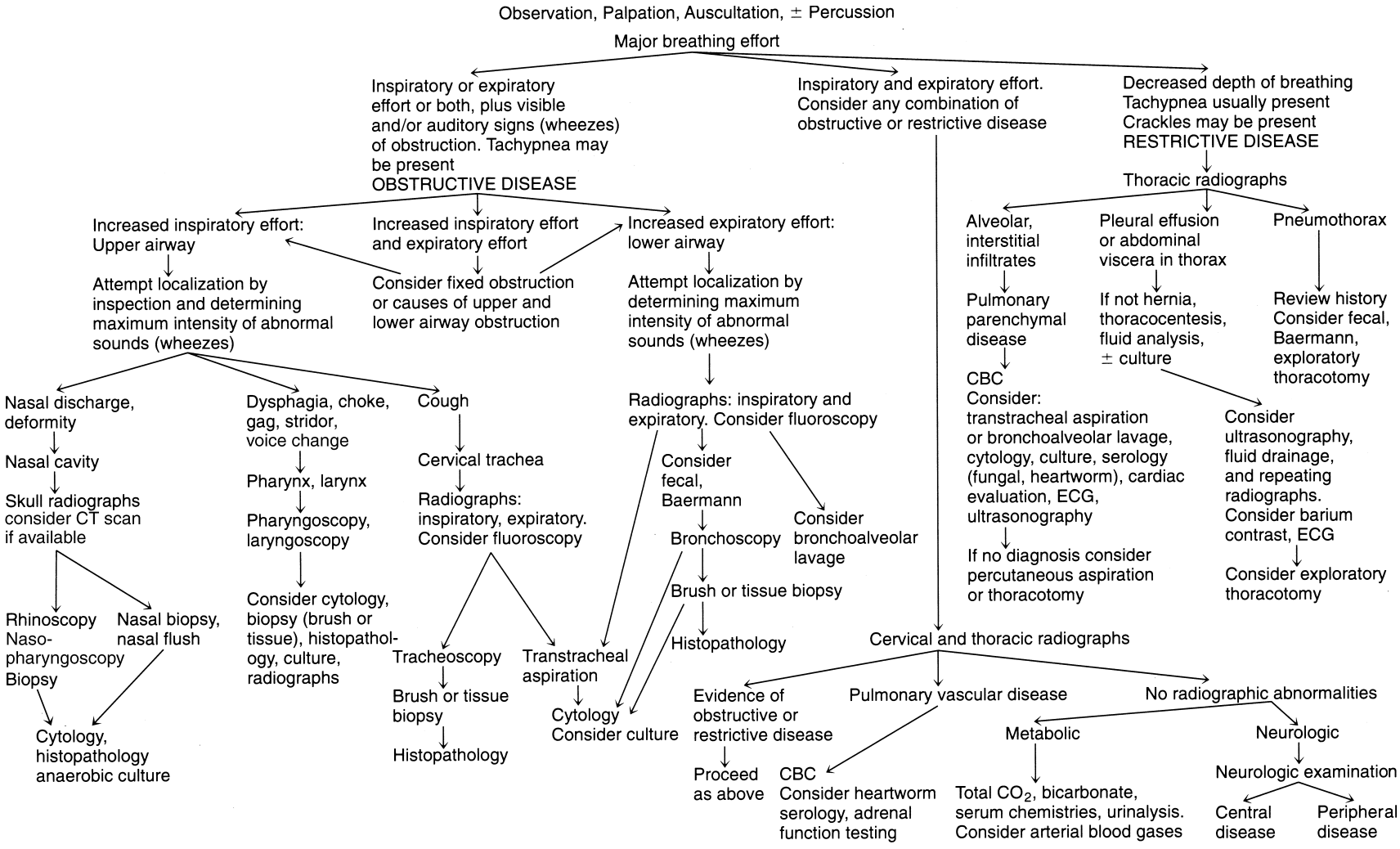

FIGURE 11-1. Approach to respiratory distress and tachypnea in dogs and cats. *CBC,* Complete blood count; *ECG,* electrocardiogram.

TABLE 11-2. Causes of Coughing in Dogs and Cats

Nasal Cavity/Sinus Disease with Postnasal Drip
See Table 11-3
Pharynx/Larynx
Trauma
Foreign body
Infection (bacterial or viral)
Neoplasia
Laryngeal paralysis (congenital or acquired)
Eversion of laryngeal saccules
Laryngeal collapse
Granulomatous laryngitis
Eosinophilic granuloma
Trachea/Lower Airway
Trauma
Foreign body
Allergy (allergic bronchitis/asthma)
Infection
  Viral (see Table 11-3)
  Bacterial *(Bordetella bronchiseptica)*
  Parasitic *(Filaroides* spp., *Oslerus osleri, Capillaria aerophilia)*
Anomalies (collapse, hypoplasia, primary ciliary dyskinesia, segmental stenosis, extraluminal compression [left atrium, tumor])
Neoplasia (osteochondral dysplasia [osteochondroma])
Degenerative disease (bronchiectasis)
Pulmonary Parenchymal Disease
Trauma
Allergy (pulmonary infiltrates with eosinophilia)
Infection
  Viral (see Table 11-3)
  Bacterial
  Fungal *(Blastomyces dermatitidis, Histoplasma capsulatum, Coccidioides immitis, Cryptococcus neoformans, Aspergillus spp.)*
  Protozoal *(Toxoplasma gondii, Pneumocystis carinii)*
  Parasitic *(Filaroides hirthi, Filaroides milksi, Dirofilaria immitis, Angiostrongylus vasorum, Paragonimus kellicotti)*
Degenerative disease (emphysema)
Neoplasia (primary or metastatic)
Noninfectious granulomatous disorders (eosinophilic pulmonary granulomatosis, pulmonary lymphomatoid granulomatosis)
Cardiovascular Disease
Pulmonary edema
Left-atrial enlargement causing bronchial compression
Thromboembolism (dirofilariasis, hyperadrenocorticism, protein-losing nephropathy, neoplasia, cardiac disease)
Mediastinal Disease (Causing Airway Compression)
Lymphosarcoma (especially cats)
Thymoma

Nasal Discharge, Sneezing, and Epistaxis

Nasal discharge and sneezing may be the result of primary nasal cavity disease or secondary to bronchopulmonary disease (e.g., pneumonia). Epistaxis may be the result of a primary nasal problem (e.g., neoplasia) or a systemic problem (e.g., coagulopathy; Kuehn and Roudebush, 1991c). Common causes of these problems are listed in Table 11-3.

TABLE 11-3. Causes of Nasal Discharge, Sneezing, and Epistaxis in Dogs and Cats

Structural Anomalies
Cleft palate
Oronasal fistula
Cricopharyngeal achalasia
Megaesophagus
Allergic/Immunologic
Allergic rhinitis
Lymphoplasmacytic rhinitis
Bleeding Disorders
Factor deficiency (congenital and acquired)
Thrombocytopenia (infectious and immune-mediated)
Vessel wall (trauma and vasculitis)
Foreign bodies/trauma
Infections
Viral: distemper, parainfluenza, adenovirus type 2 (dogs); herpesvirus, calicivirus (cats)
Bacterial: including dental disease, chronic feline rhinosinusitis
Fungal: *Aspergillus* spp., *Penicillium* spp., *Cryptococcus neoformans, Rhinosporidium seeberi*; other opportunistic fungi are rare (e.g., *Trichosporon*)
Rickettsial (*Ehrlichia canis*, Rocky Mountain spotted fever)
Parasitic (*Pneumonyssoides caninum, Linguatula serrata, Capillaria aerophilia, Syngamus ierei, Cuterebra* spp.)
Other (*Chlamydia* spp.)
Neoplasia/Polyps
Carcinomas, sarcomas, transmissible venereal tumor
Polyp (nasopharyngeal in cats)

When epistaxis occurs, evaluation for coagulopathy should be the initial diagnostic step (see Chapter 5). Radiography or computed tomography (CT) of the nasal cavity is usually performed next (Figure 11-2) but is seldom diagnostic in acute disease. If a mass lesion or bone lysis is identified, biopsy via the naris is indicated. Rhinoscopy to look for foreign objects is next and can be performed while a patient is anesthetized for radiographs. Direct examination may reveal adult *Pneumonyssoides caninum*. Serologic testing for nasal aspergillosis can be falsely negative and should not be relied on for a diagnosis (see Chapter 15). Direct examination of the nasal cavity (dorsal and ventral meatus) can be performed with rigid or flexible scopes (see Rhinoscopy). The nasopharynx and posterior portion of the nasal cavity can be visualized with a dental mirror and penlight or more efficiently with a flexible endoscope. Endoscopy of the anterior portion of the nasal cavity in small dogs and cats is limited by the endoscope's diameter. Nasal lavage is rarely diagnostic. Bacterial culture is not routinely recommended; interpretation is difficult because of the large normal

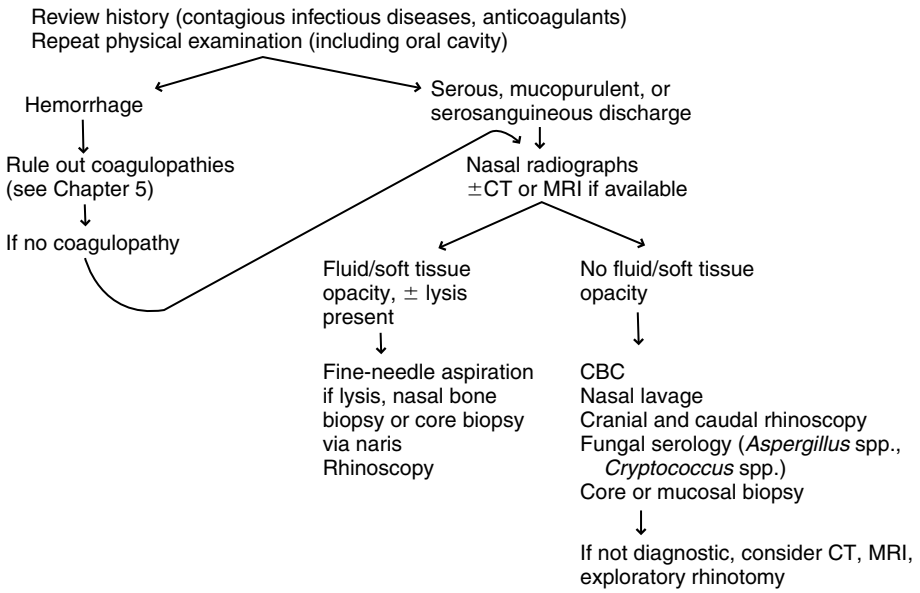

FIGURE 11-2. Approach to nasal discharge and epistaxis in dogs and cats. *CBC,* Complete blood count; *CT,* computed tomography; *MRI,* magnetic resonance imaging.

bacterial population of the nasal cavity (see Chapter 15). Fungal culture for aspergillosis can have both false-positive and false-negative results (see Chapter 15). Cultures for fungi are generally more reliable if performed on nasal biopsy specimens rather than nasal cavity swabs. Nasal biopsy samples can be obtained with alligator-type clamshell instruments, with uterine biopsy forceps, or by catheter core procedures (described later). If these procedures are not diagnostic and the condition persists, CT or exploratory rhinotomy should be considered.

NASAL RADIOGRAPHY

Common Indications • Nasal radiography is indicated for chronic nasal discharge, epistaxis, severe acute undiagnosed sneezing, facial deformity, nasal obstruction, or pawing at the face or nose (see Table 11-3).

Advantages • Nasal radiography is noninvasive. Neoplasia and aspergillosis often cause bone lysis, which is seldom present in other disorders.

Disadvantages • General anesthesia is required, and excessive fluid (e.g., nasal hemorrhage, exudate) may obscure soft tissue abnormalities. In addition, the procedure provides low resolution of fine detail (except for high-detail dental films in cats and small dogs).

Readers are referred to a radiology text for additional information. CT or magnetic resonance imaging (MRI) are the preferred imaging modalities and provide superior detail for evaluating the nasal cavity (Codner et al, 1993).

COMPUTERIZED TOMOGRAPHY (CT)

Common Indications • CT has the same indications as nasal radiography. It is essential for planning radiation therapy for nasal tumors and highly recommended before topical clotrimazole therapy for nasal aspergillosis (to ensure that the cribiform plate has not been eroded).

Advantages • CT provides much more detail than radiographs. CT is excellent at finding "caverns" where aspergillosis has caused destruction and loss of turbinate structures, and it is far superior at finding tumors. CT readily detects involvement of the calvarium and cribiform plate in both aspergillosis and tumors.

Disadvantages • The need for specialized equipment and the greater cost compared with radiographs are the major drawbacks. Occasionally, contrast media are needed to

distinguish between non-enhancing soft tissue density due to nasal discharge and enhancing density due to tumor or inflammation. Readers are referred to a radiology text for additional information.

RHINOSCOPY

Occasional Indications • Rhinoscopy is generally performed for the same reasons as nasal radiography.

Advantages • Rhinoscopy is relatively noninvasive, may be done after nasal imaging during the same anesthetic procedure, and may provide definitive diagnosis. It is especially useful for diagnosing nasal aspergillosis.

Disadvantages • Anesthesia is required. If an otoscope cone is used, only the rostral nares can be visualized. Even with a fiberscope, arthroscope, or cystoscope, overall visualization is limited, necessitating a careful, methodic examination that does not always allow diagnosis. Copious nasal discharge or hemorrhage will obstruct visualization of nasal structures. In small dogs, rhinoscopy is difficult unless an arthroscope is available. Care must be taken to avoid causing hemorrhage, which can obscure the field of view.

Procedure • Radiographs should be obtained first. Next, posterior rhinoscopy is performed by retracting the soft palate with an ovariectomy hook and visualizing the area with a dental mirror and nasopharyngeal illuminator, or a flexible endoscope. Anterior rhinoscopy is then performed using an otoscope, nasal speculum, flexible endoscope, arthroscope, or cystoscope. Tissue or brush biopsies can be obtained for cytologic analysis, histopathologic examination, or culture.

Analysis and Interpretation • See Nasal Core Biopsy.

NASAL LAVAGE

Occasional Indications • Nasal lavage can be performed for the same reasons as nasal radiography.

Advantages • It is less invasive and produces fewer complications than core biopsy.

Disadvantage • It is seldom diagnostic.

Procedure • Under general anesthesia with endotracheal intubation, the nasopharynx is packed off with gauze sponges and the nasal cavity is vigorously lavaged with lactated Ringer's via a soft rubber tube.* The fluid is recovered in a dish placed at the nares. A foreign body may occasionally be dislodged and recovered from the naris or gauze sponges in the nasopharynx.

Analysis • Recovered fluid is centrifuged, and the sediment is stained and examined. A Wright's-Giemsa or Gram's stain is preferred when looking for organisms (e.g., *Cryptococcus* spp.). If lavaged material appears to be an exudate, it can be cultured for fungi. Bacterial culture is rarely useful. Direct examination of lavage fluid may reveal adult or larval *P. caninum*.

Interpretation • In allergic rhinitis, many eosinophils may be visible (see Nasal Core Biopsy for additional interpretation of nasal specimens).

NASAL CORE BIOPSY

Common Indications • Indications are the same as for nasal lavage. Core biopsy is often performed because nasal radiographs suggest a mass lesion or bone lysis. In the absence of these, the procedure may be performed but is only occasionally diagnostic.

Advantage • Tissue can be obtained for histopathologic evaluation.

Disadvantages • Procedure often causes bleeding. Although unlikely, penetration of the cribriform plate is possible if adequate care is not taken.

Procedure • If the animal's coagulation status is questionable, a platelet count, mucosal bleeding time, and activated clotting time should be performed (see Chapter 5). Nasal core biopsy is performed under general anesthesia with endotracheal intubation. The nasopharynx is packed off as for nasal lavage. The biopsy instrument can be either a large-bore tube that covers one type of peripheral vein indwelling catheter† (medium or

*Rob-Nel catheter, Sherwood Medical, St. Louis, MO.
†Sovereign indwelling catheter, Monoject Division of Sherwood Medical, St. Louis, MO.

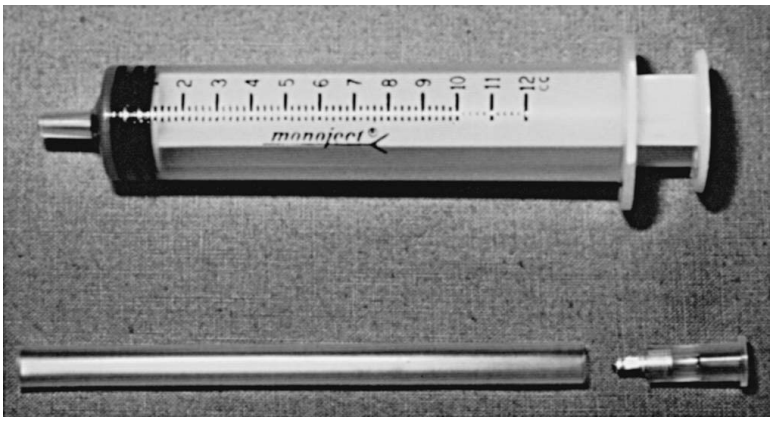

FIGURE 11-3. Plastic tube and needle hub used to obtain nasal biopsy specimen.

large dog) or an 8-gauge polypropylene urinary catheter* (small dog or cat) cut at 45 degrees about 12 to 15 cm from the end that attaches to the syringe. If the large-bore tube is used, it is attached to the syringe by the cutoff hub of the needle supplied with the catheter (Figure 11-3). The distance from the naris to the medial canthus of the eye is marked on the tube, because this approximates the distance to the cribriform plate. The tube is advanced into affected tissue, suction is applied, and the tube containing a core of tissue is withdrawn. If a cutoff 8-gauge catheter is used, it is marked as for the tube and moved vigorously back and forth to dislodge tissue; one must be careful not to penetrate the cribriform plate. A syringe containing lactated Ringer's (35 ml in a large dog, 10 ml in a cat or small dog) is attached, and dislodged tissue is flushed out through the naris or into the nasopharynx. In addition, material can be obtained by aspiration. Alternatively, bone curettes, alligator-type clamshell forceps, or uterine biopsy forceps may be used instead of a tube or catheter.

A portion of the biopsy specimen can be submitted for fungal culture (see Chapter 15). Impression smears can be made for cytologic examination, and the remaining tissue can be submitted for histopathologic evaluation. Bleeding from the naris after biopsy is expected but usually subsides within 30 minutes. Occasionally, bleeding is profuse, prolonged, or both, in which case the affected area can be packed with cotton-tipped applicator sticks dipped in dilute (1:10,000) epinephrine solution while the animal is maintained under anesthesia until bleeding stops. Alternatively, nasal tampons* may be used. *Caution:* The tube must not be advanced beyond the level of the medial canthus because of potential cribriform plate perforation.

Interpretation of Impression Cytology

Infection • Because both the healthy and diseased nasal cavity contain various bacteria (see Chapter 15), identification of bacteria is rarely significant. *Aspergillus* spp. and *Penicillium* spp. can occasionally be recovered from the nasal cavity of normal animals, as well as from some with other disorders (e.g., neoplasia); therefore, a diagnosis of aspergillosis or penicilliosis must be confirmed by histopathologic evaluation. Finding *Cryptococcus* spp. in a cat with chronic nasal discharge is usually diagnostic (Figure 11-4; Color Plate 4E); however, *Cryptococcus* spp. occasionally is cultured from nasal washings of normal dogs and cats (Malik et al, 1997).

Neoplasia • The most common nasal tumors are adenocarcinomas and carcinomas (Figure 11-5), although round cell tumors (i.e., transmissible venereal tumor, mast cell tumor, lymphosarcoma) occasionally occur. Other malignant mesenchymal tissue tumors (e.g., fibrosarcoma, osteosarcoma) may occur but are less exfoliative and more difficult to diagnose cytologically (see the discussion of cytologic evaluation of neoplastic cells in Chapter 16).

Hemorrhage • In nasal hemorrhage, proportions of erythrocytes and leukocytes are approximately equivalent to whole blood.

*Polypropylene catheter, Monoject Division of Sherwood Medical, St. Louis, MO.

*Merocel Standard Nasal Dressing with drawstring, Medtronic Xomed, Jacksonville, FL.

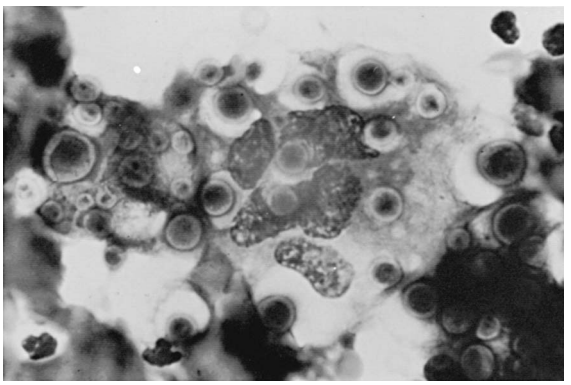

FIGURE 11-4. *Cryptococcus neoformans* from an impression smear from a nasal biopsy specimen. *(Courtesy of Dr. S.D. Gaunt, Louisiana State University.)*

NASAL MUCOSAL BIOPSY

Occasional Indications • Nasal mucosal biopsy is indicated occasionally if the patient has undiagnosed nasal discharge without radiographic evidence of masses or bone lysis. Two diseases that may be diagnosed by this method are (1) lymphocytic-plasmacytic rhinitis and (2) primary ciliary dyskinesia (i.e., immotile cilia syndrome), although the latter requires electron microscopy to be definitive. The procedure can also be used instead of core biopsy for nasal masses or lytic lesions.

Procedure • Specimens are obtained with alligator biopsy forceps, uterine biopsy forceps, or a bone curette, fixed in formalin (or other appropriate fixative if electron microscopy is desired), and submitted.

NASAL FINE-NEEDLE ASPIRATION BIOPSY

Occasional Indications • Nasal fine-needle aspiration biopsy is performed if the patient has nasal bone destruction that permits a needle to be introduced into the nasal cavity without going through the naris.

Advantages • Anesthesia is not required, and the procedure is minimally invasive.

Disadvantage • Some tumors (e.g., mesenchymal) are poorly exfoliative.

Procedure • The area of bone lysis is identified by palpation or by nasal radiographs. A 23- or 25-gauge needle is inserted through the lytic area of bone, and aspiration is performed. Specimens are submitted for cytologic examination.

Interpretation • See Interpretation of Impression Cytology under Nasal Core Biopsy, earlier.

EXPLORATORY RHINOTOMY

Occasional Indications • Exploratory rhinotomy can be performed on patients with sneezing, nasal discharge, or epistaxis

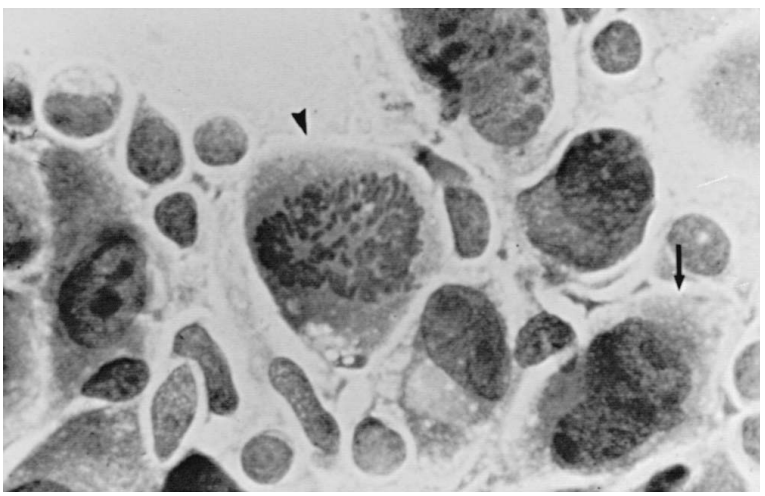

FIGURE 11-5. Carcinoma cell *(arrow)* from an impression smear of a nasal biopsy. Note the mitotic figure *(arrowhead)*. *(Courtesy of Dr. C.L. Barton, Texas A&M University.)*

when the cause has not been determined by any of the previously discussed procedures. If available, CT should be performed before rhinotomy.

Advantages • It allows excellent visualization, biopsy, and culture of the nasal cavity, as well as identification of foreign bodies.

Disadvantage • It is an invasive, painful procedure.

Procedure • Readers are referred to a surgical text for a procedure description. Impression smears for cytologic examination are made from tissue samples. Tissue is cultured for fungi and fixed in formalin to be submitted for histopathologic evaluation.

SEROLOGY FOR NASAL FUNGAL DISORDERS

Occasional Indications • Serology for nasal fungal disorders is done for patients with chronic, undiagnosed nasal discharge that may be the result of aspergillosis or cryptococcosis (and rarely other fungi; see Chapter 15).

COAGULATION TESTS FOR EPISTAXIS

Occasional Indications • Coagulation tests for epistaxis are important in patients with undiagnosed nasal cavity hemorrhage, especially before surgery or aggressive biopsy (see Chapter 5). Chronic unilateral nasal hemorrhage without systemic signs of hemorrhage is usually caused by primary nasal disease; coagulation tests are recommended but not mandated. Acute bilateral hemorrhage necessitates mucosal bleeding time and platelet count, however. Coagulation times are also appropriate.

LARYNGEAL AND PHARYNGEAL EXAMINATION

Occasional Indications • Laryngeal and pharyngeal examination are important for patients with stridor or gagging suggestive of an upper-airway obstructive disorder (e.g., nasopharyngeal polyp, laryngeal paralysis) or foreign body.

Advantage • It permits definitive diagnosis and treatment if polyps, laryngeal paralysis, or foreign bodies are present.

Disadvantage • It requires appropriate anesthesia.

Procedure • Under a light plane of anesthesia, movement of the laryngeal cartilages is observed. The corniculate processes of the arytenoid cartilages and vocal folds normally should abduct with inspiration and passively adduct with expiration. With laryngeal paralysis, these structures do not abduct with inspiration but remain in a partially adducted position or collapse (i.e., adduct) on forceful inspiration. The pharynx and nasopharynx are examined as described for posterior rhinoscopy.

TRACHEAL AND THORACIC RADIOGRAPHY

Common Indications • Tracheal and thoracic radiography are important as early diagnostics for patients with chronic or severe cough or other bronchopulmonary disease.

Advantages • It is noninvasive and often localizes problems.

Disadvantage • It is rarely of value in acute inflammatory disorders (e.g., viral tracheobronchitis) or thromboembolism not caused by dirofilariasis (LaRue and Murtaugh, 1990).

Procedure and Interpretation • The reader is referred to a radiology text for additional information. Evaluation of both right and left lateral views improves visualization of pulmonary masses (Steyn and Green, 1990).

TRANSTRACHEAL ASPIRATION

Common Indications • Indications for transtracheal aspiration are generally the same as for thoracic radiographs.

Advantages • It is relatively noninvasive, yet samples the tracheobronchial tree without anesthesia.

Disadvantages • Although uncommon, complications can include subcutaneous (SC) emphysema originating at the site of needle

penetration, esophageal perforation, hemorrhage, and lower-airway catheter stylet trauma. Samples may not accurately reflect lower airway or lung disease (e.g., bronchiolitis, interstitial pneumonia). It is contraindicated in patients with severe respiratory distress, because restraint can worsen the distress and cause death. Severe coagulopathy is a relative contraindication.

Procedure • Dogs often tolerate TTA without sedation, but tranquilizers (e.g., acepromazine) can be used. For cats, sedation (e.g., 1 to 2 mg/kg ketamine intravenously [IV]) is routine; oxygen should be available if respiratory distress occurs. The patient is restrained in sternal recumbency. After clipping and surgical preparation of the skin over the larynx, a bleb of lidocaine is injected over the cricothyroid membrane. A through-the-needle type of catheter* (20-gauge for cats and small dogs, 16-gauge for medium-sized and large dogs) is inserted through the cricothyroid membrane and advanced to approximately the level of the mainstem bronchi. Alternatively, a sterile 3.5-French polypropylene urinary catheter can be inserted through a 14-gauge needle in large dogs. In anesthetized animals, the catheter can be inserted through a sterile endotracheal tube (recommended for cats if anesthesia is tolerated).

Depending on the animal's size, 0.5 to 1.0 ml/kg of sterile lactated Ringer's solution (0.9% saline is acceptable but causes more cellular distortion) is injected into the catheter. After the animal coughs, aspiration is performed. Chest coupage after fluid instillation may improve recovery of debris from the airways. Usually only a small amount of injected material is recovered. If a low-pressure (i.e., <5 mm Hg) suction pump is available, yield is higher. Using the suction pump method, material can be aspirated into a suction trap.** Multiple aliquots of lactated Ringer's solution can be injected until a sample is obtained. Aliquots of aspirate may be submitted for cytologic analysis and aerobic, anaerobic, or fungal culture. The use of "roll preparations" of the cellular pellet of spun samples is advised, because cellular disruption often results if unspun fluid samples cannot reach the laboratory within 1 hour of collection. To make a roll preparation, the wooden end of a cotton-tipped applicator stick is used to gently roll cells from the pellet onto clean glass slides. The decision to culture is based on cytologic findings (see the following text under Interpretation).

Interpretation • Occasional ciliated columnar or cuboidal epithelial cells, occasional undifferentiated macrophages with few or no vacuoles, rare neutrophils, and small amounts of mucus are normal. Some normal epithelial cells may appear smudged or lack cilia because of trauma during sample preparation. TTA aspirates from animals with bronchopulmonary disease may be classified as mucopurulent inflammation, nonpurulent inflammation, neoplastic, or hemorrhagic (see Chapter 16).

Mucopurulent Inflammation • This aspirate is a mixture of neutrophils (see the discussion of neutrophilic inflammation in Chapter 16) and abundant mucus, often plus a few macrophages (Figure 11-6). In mucopurulent inflammation, the mucoid material may stain basophilic (blue) or become eosinophilic (pink) as the inflammation becomes more severe. A thorough search for bacteria (especially intracellular) should be made if degenerate neutrophils are seen (Figure 11-7). Causes of mucopurulent inflammation include bacterial, fungal, viral, mycoplasmal, and protozoal infection, as well as chronic bronchitis, tumors, foreign bodies, and aspiration (Padrid et al, 1991).

A portion of the TTA sample should be placed in a culture transport medium (see Chapter 15) before cytologic evaluation. If mucopurulent inflammation is found, this portion should be submitted for culture (see Chapter 15).

Nonpurulent Inflammation • This aspirate includes a higher percentage of macrophages (see the discussion of granulomatous and pyogranulomatous inflammation in Chapter 16), eosinophils (see the discussion of eosinophilic inflammation in Chapter 16), or both than found in mucopurulent exudates. Nonpurulent exudates may contain a predominance of eosinophils or macrophages or a mixture of eosinophils, macrophages, and neutrophils. Eosinophilic inflammation (Figure 11-8) suggests hypersensitivity caused by inhaled allergens, parasites, or eosinophilic

*Intracath, Deseret Medical Inc., Sandy, UT.
**Dee Lee suction catheter, American Hospital Supply, McGraw Park, IL.

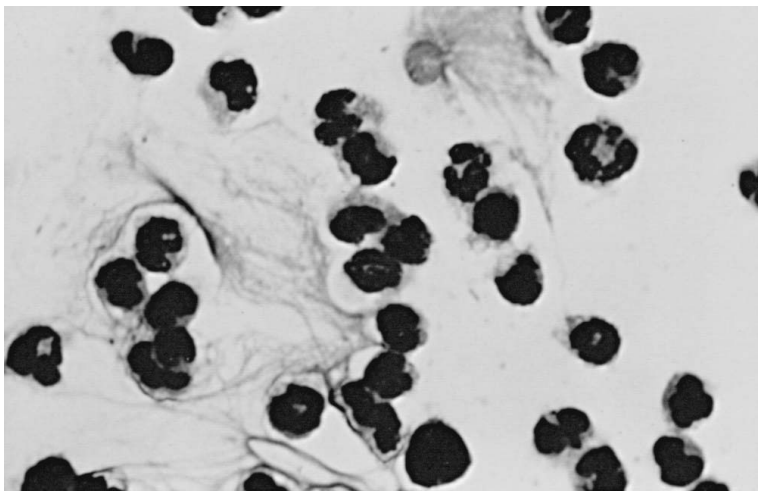

FIGURE 11-6. Mucopurulent inflammation from a transtracheal aspirate of a dog with chronic obstructive pulmonary disease associated with collapsed trachea. Note the large numbers of nondegenerate neutrophils and abundant mucus.

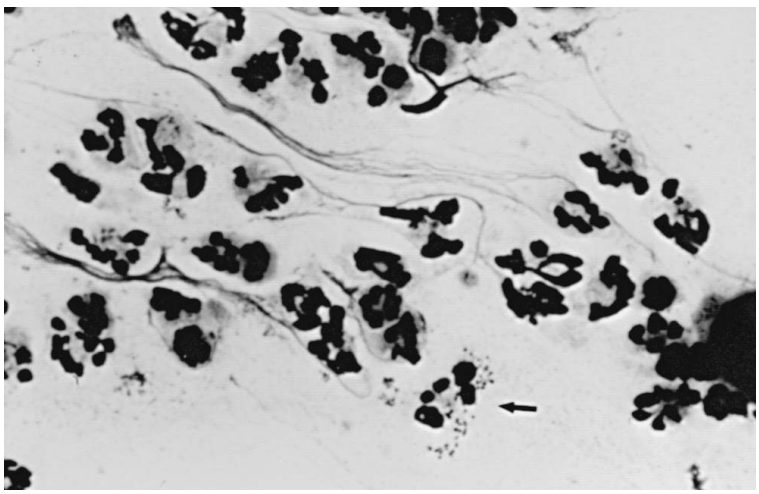

FIGURE 11-7. Septic inflammation in a transtracheal aspiration (TTA) specimen from a dog with bacterial pneumonia. Note the large number of degenerate neutrophils, some containing intracellular bacteria *(arrow)*. *(Courtesy of Dr. P.S. MacWilliams, University of Wisconsin.)*

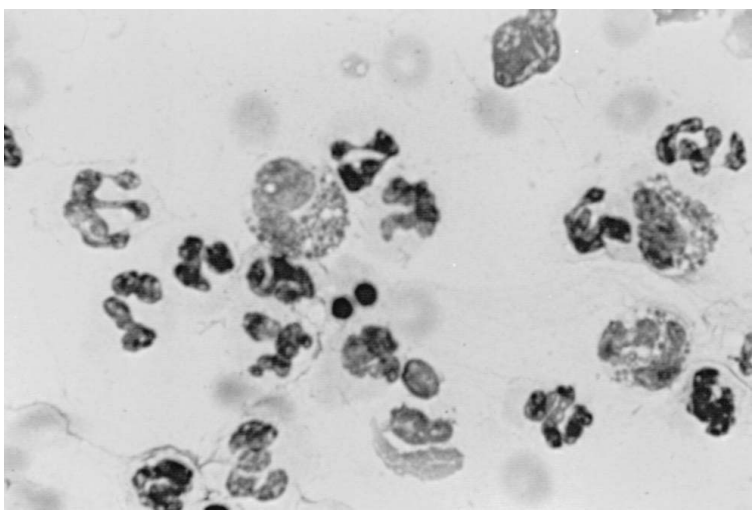

FIGURE 11-8. Eosinophilic inflammation in a transtracheal aspiration (TTA) specimen from a cat with asthma. *(Courtesy of Dr. K.A. Gossett, Louisiana State University.)*

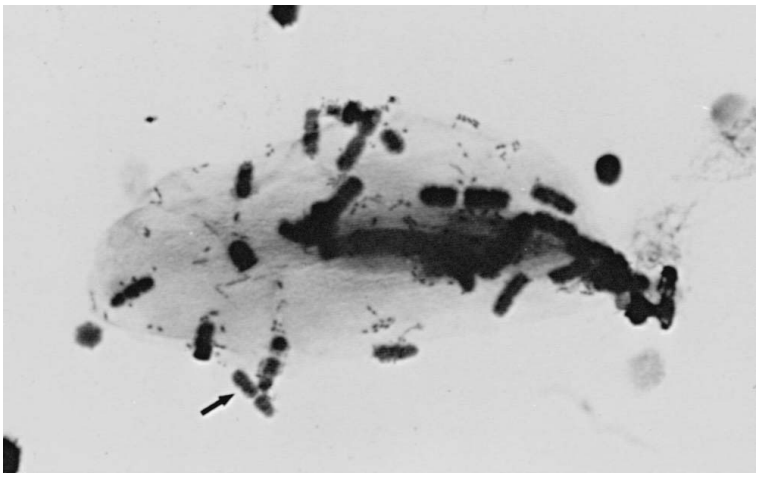

FIGURE 11-9. *Simonsiella* spp. *(arrow)* and a squamous epithelial cell in a transtracheal aspiration specimen (TTA) from a dog. *(Courtesy of Dr. D. Baker, Colorado State University.)*

pulmonary granulomatosis (Kuehn and Roudebush, 1991d). Parasitic causes include *D. immitis, Aelurostrongylus abstrusus, Capillaria aerophilia, Oslerus osleri, Filaroides* spp., *Paragonimus kellicotti*, or migrating parasites such as *Toxocara* spp. or *Ancylostoma* spp. Small numbers of mast cells are common in eosinophilic inflammation. A predominance of differentiated macrophages (i.e., larger macrophages with abundant cytoplasm and numerous cytoplasmic vacuoles) suggests subacute to chronic disease, such as granulomatous pneumonia caused by fungi or lipid. Fungal organisms are rarely recovered, because they tend to be interstitial, not in the airways.

Other Cells in Transtracheal Aspirates • Reactive epithelial cells may be found with any inflammatory process, especially in cats. The cytoplasm is more basophilic (blue) than normal epithelial cells, and nuclei have fine chromatin and visible nucleoli. Cells may be single or clustered.

Goblet cells are occasionally visible in inflammatory disease. They contain granules of intracellular mucus and often occur in conjunction with abundant extracellular mucus.

Neoplastic cells are occasionally recovered from animals with primary lung tumors, particularly adenocarcinomas. Primary lung tumors, however, are much less common than metastatic tumors. Because of their interstitial location, cells from metastatic pulmonary tumors are rare in TTA specimens.

Aspirated material from the oral cavity is suggested by squamous epithelial cells (which may be coated with bacteria) or certain large bacteria such as *Simonsiella* spp. (Figure 11-9).

Lymphocytes may be visible in acute viral tracheobronchitis, and lymphocytes plus plasma cells may be visible in chronic, progressive, septic bronchopneumonitis; sterile bronchopneumonitis; or pulmonary lymphoid granulomatosis (Berry et al, 1990).

Ova of *C. aerophilia, P. kellicotti*, and *Filaroides hirthi* occasionally are found, as are larvae of *O. osleri, Crenosoma vulpis, A. abstrusus, Toxocara canis, T. cati*, and *Strongyloides stercoralis*, as well as microfilariae of *D. immitis*.

Anthracotic pigment appears as dense black granules within macrophages and is an incidental finding in dogs in industrial areas.

BRONCHOALVEOLAR LAVAGE

Common Indications • Indications for bronchoalveolar lavage are generally the same as for TTA.

Procedure • BAL is an invasive technique, requires general anesthesia, and causes temporary respiratory compromise (Hawkins and DeNicola, 1989; Hawkins, DeNicola, and Kuehn, 1990). Although not technically difficult, practice is required to acquire confidence and skill in performing BAL. The procedure can be performed through an endotracheal tube; however, endoscopic BAL is recommended because of improved site selectivity for collection of specimens and enhanced retrieval of lavage fluid.

Animals undergoing BAL should receive atropine as a preanesthetic and be anesthetized with ketamine/acepromazine, or ketamine/diazepam IV, isoflurane tank induction (cats), or with other IV short-acting (e.g., propofol) anesthetic agents (dogs). Additional anesthetic can be given IV if needed to maintain anesthesia. BAL may be performed directly through a sterile endotracheal tube (4 mm internal diameter) in cats and very small dogs. The endotracheal tube is placed rostral to the carina, and the cuff is inflated. Oxygen (100% O_2) is administered for 1 to 2 minutes before lavage. After preoxygenation, the patient is placed in lateral recumbency with the most affected side down and a syringe is attached to the endotracheal tube. Three aliquots (5 ml/kg) of warm, sterile lactated Ringer's solution (0.9% saline is acceptable but causes more cellular distortion) are gently infused into the lung and immediately retrieved with gentle suction. Elevating the patient's hindquarters improves retrieval of BAL fluid. Each aliquot is kept in a separate syringe for analysis. After the procedure, O_2 (100%) is administered continuously until the animal is fully awake and breathing is no longer suppressed.

If an endoscope is used, an endotracheal tube is not necessary. Rather, the endoscope can be passed into the trachea while O_2 is delivered through the endoscopic biopsy channel (be careful to avoid barotrauma) or through an adapter adjacent to the endoscope. In larger dogs, the endoscope can be passed through an endotracheal tube and O_2 and inhalant anesthesia administered around the endoscope with a T-adapter. The distal end of the endoscope is "wedged" in a main stem bronchus or airway of interest. Three aliquots (i.e., 2 ml/kg) of warmed, sterile lactated Ringer's solution are flushed through the endoscopic biopsy channel and immediately retrieved as previously described. Pre- and post-BAL oxygenation is performed as previously described.

BAL fluid often appears foamy because of pulmonary surfactant recovered by the BAL procedure. Unspun BAL specimens should be transferred immediately to the laboratory for analysis. If rapid transport of BAL fluid to the laboratory is impossible, cytologic specimens should be prepared immediately by cytocentrifugation or roll preparations.* Roll preparations are made from the cell pellet resulting from centrifugation. The wooden end of a cotton-tipped applicator stick is used to gently roll cells from the pellet onto clean glass slides. These consistently produce excellent cytologic specimens.

The cellular character of BAL preparations is vastly different from TTA specimens. The predominant cell type is the alveolar macrophage; however, large numbers of eosinophils (up to 30%) can be present in clinically normal cats (Padrid et al, 1991). Other cell types and infectious organisms (e.g., yeast, fungal hyphae, bacteria) may be present, depending on the patient's disease. Interpretation is similar to that described for TTA (Hawkins and DeNicola, 1990).

Because of inevitable oral contamination of instruments used for BAL procedures, BAL specimens are not useful for accurate bacteriologic culture (unless sterilization of the scope and associated equipment is feasible). Specimens for culture should be collected by TTA or with a guarded swab.

TRACHEOBRONCHOSCOPY

Occasional Indications • Tracheobronchoscopy is primarily performed in patients with persistent undiagnosed coughing, hemoptysis, or a suspected obstructive lesion; whenever direct visualization of larger airways is required to look for obstruction or collapse; or to selectively sample an area of the tracheobronchial tree. It is the procedure of choice to diagnose collapsed trachea if radiography or fluoroscopy has not established the diagnosis. Flexible bronchoscopes are preferred.

Advantages • It provides direct visualization of major airways and allows biopsy of specific sites. This is the technique of choice to diagnose *O. osleri* infection. Cytologic specimens obtained by brush biopsy are usually superior to those obtained by TTA. Pulmonary biopsy can be performed via a transbronchial biopsy.

Disadvantages • It requires general anesthesia; pulmonary biopsy has the potential risk of tracheal rupture (especially in cats), pneumomediastinum, pneumothorax, and pulmonary hemorrhage. If oxygen is being insufflated through the biopsy channel, care must be taken to avoid barotrauma.

*Cytocentrifuge II, Shandon Southern Instruments, Sewickley, PA.

Procedure • The endoscope is either passed through an endotracheal tube (large dog) or directly into the trachea (small dog or cat). A thorough systematic examination is made of all accessible parts of the tracheobronchial tree (Rha and Mahony, 1999).

Interpretation • In collapse of the trachea, mainstem bronchus, or bronchi, severity of the lesion is assessed. Other lesions are visualized and may be sampled by either brush or endoscopic forceps. Specimens obtained may be submitted for bacteriologic culture or for cytologic and histopathologic examination.

FECAL EXAMINATION

Occasional Indications • Fecal examination can be helpful for patients with undiagnosed coughing or dyspnea, particularly if unexplained eosinophilia is present or if radiographic signs suggest pulmonary parasitism.

Advantage • It is noninvasive.

Disadvantage • Ova of some pulmonary parasites (e.g., *F. hirthi*) are not reliably recovered.

Procedure • Fecal flotation is performed using zinc sulfate solution (see Chapter 9).

Interpretation • Ova of *C. aerophilia* (Figure 11-10), *F. hirthi*, *Eucoleus boehmi*, and *P. kellicotti* (Figure 11-11) may be detected.

BAERMANN'S FUNNEL APPARATUS

Occasional Indications • Use of a Baermann's funnel apparatus can be used for the same reasons as for fecal flotation for respiratory parasites.

Advantage • It is noninvasive.

Disadvantages • It is inconvenient and cumbersome; some parasites (e.g., *O. osleri*) shed larvae erratically.

Procedure • Fresh feces are placed on a cheesecloth in a strainer and then in the Baermann's apparatus (a funnel with a clamped rubber tube at the stem end). Water is added to the funnel to cover the feces, which are then broken up into small pieces. After a few hours, a small aliquot of water is drained through the rubber tube and examined microscopically for larvae.

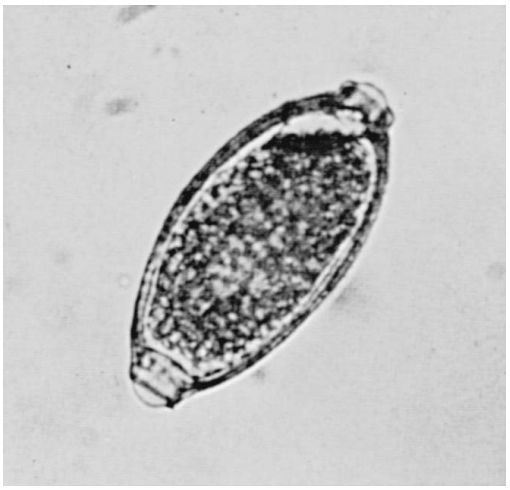

FIGURE 11-10. *Capillaria aerophilia* egg in a fecal specimen from a dog with chronic cough. *(Courtesy of Dr. J.B. Malone, Louisiana State University.)*

Interpretation • Larvae of *Filaroides milksi*, *F. hirthi*, *O. osleri*, *C. vulpis*, *A. abstrusus*, and *S. stercoralis* may be identified, but a negative test result does not eliminate these parasites. The method of choice to diagnose *O. osleri* infection is bronchoscopy.

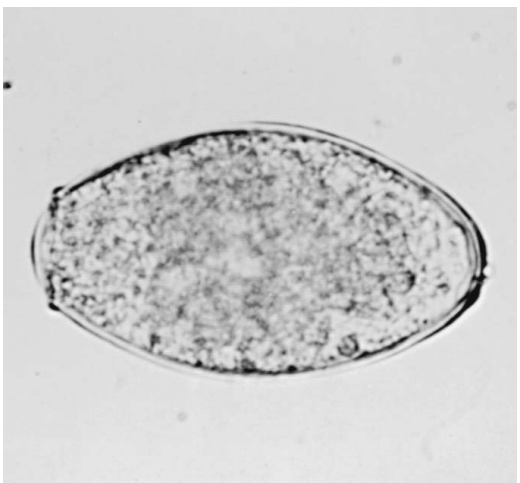

FIGURE 11-11. *Paragonimus kellicotti* egg in a fecal specimen from a cat with chronic cough. *(Courtesy of Dr. J.B. Malone, Louisiana State University.)*

PULMONARY ASPIRATION BIOPSY

Occasional Indications • Pulmonary aspiration biopsy is primarily used in patients with masses, diffuse infiltrative disease, and to procure material for culture.

Advantage • It samples pulmonary parenchyma without thoracotomy.

Disadvantages • Possible complications include pneumothorax, pulmonary hemorrhage, hemoptysis, and (if the myocardium is inadvertently penetrated) cardiac arrhythmias, any of which may rarely cause death.

Contraindications • It is contraindicated in uncooperative patients and those with thrombocytopenia, bleeding disorders, severe uncontrolled coughing, and pulmonary bullae or cysts.

Procedure • Coagulation status should be screened (see Chapter 5) with platelet count and mucosal bleeding time. For diffuse pulmonary disease, the recommended aspiration site is between the seventh and ninth intercostal spaces, two thirds of the distance from the costochondral junction to the vertebral bodies. A 25-gauge hypodermic or spinal needle* with stylet removed and 12 ml syringe are used. After clipping and surgical preparation of the skin, the needle is attached to the syringe and inserted into the pulmonary parenchyma, and aspiration is performed. The procedure should be done quickly, actual aspiration not taking more than a few seconds. Very little material is usually aspirated, and what is present typically remains in the needle hub. The needle should be removed from the syringe, air introduced into the syringe, the needle reattached, and the aspirated material quickly "blown" onto a clean glass slide. Another clean slide is used to prepare a horizontal "pull-apart" specimen for cytologic examination. Pulmonary aspiration should be performed early in the day so that the patient can be monitored for dyspnea caused by pneumothorax, pulmonary hemorrhage, or hemoptysis. Ultrasonography is generally not useful for evaluating the pulmonary parenchyma. If a mass lesion is present, however, ultrasonographic or fluoroscopic guidance may help in needle placement.

*Spinal needle, Becton-Dickinson, Rutherford, NJ.

Larger lung tissue specimens for histopathologic examination can be obtained with a similar technique using a modified Menghini's aspiration biopsy needle* (Teske et al, 1991) or Bard Monopty needle† (Bauer, 2000). Complications are similar to those for pulmonary aspiration biopsy.

Interpretation • Fine-needle aspiration biopsy specimens are examined cytologically and classified as inflammatory, neoplastic, or hemorrhagic (see Chapter 16). Larger specimens are submitted for histopathologic examination.

Inflammatory • Neutrophils, monocytes, or eosinophils predominate. Abundant erythrocytes are common. In fungal pneumonia, especially if caused by blastomycosis or histoplasmosis, free or engulfed yeasts may be visible (Figure 11-12). The number of eosinophils can be increased in aspirates from animals with pulmonary infiltrates with eosinophilia, as well as other hypersensitivities and parasitism.

Neoplastic • Neoplastic cells occasionally are visible. Malignant epithelial cells tend to appear in clusters (e.g., rafts), and inflammatory cells may be present.

Hemorrhagic • Hemorrhage frequently occurs and is usually iatrogenic.

Parasitic • Adult *F. hirthi* organisms are rarely recovered.

SEROLOGY FOR PULMONARY DISEASES

In histoplasmosis, blastomycosis, cryptococcosis, and coccidioidomycosis, finding organisms is diagnostic. Pulmonary disease caused by these organisms is usually interstitial, and TTA rarely demonstrates the organism. Positive serologic results, particularly for coccidioidomycosis, blastomycosis, and cryptococcosis, may establish a tentative diagnosis when TTA, BAL, and pulmonary aspiration biopsy do not (check characteristics of individual tests in Chapter 15). Serologic testing for *Toxoplasma gondii* is rarely indicated in patients with pulmonary disease.

*Modified Menghini Biopsy needle, Becton-Dickinson, Rutherford, NJ.
†Monopty biopsy needle, C.R. Bard, Inc., Murray Hill, NJ.

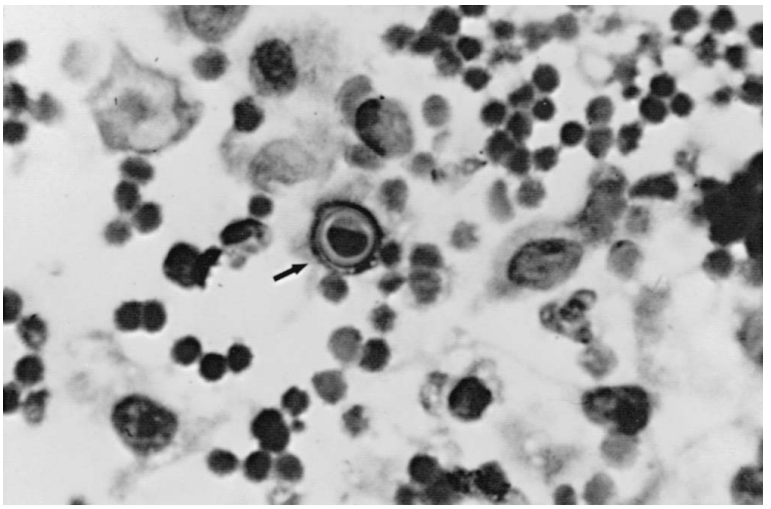

FIGURE 11-12. *Blastomyces dermatitidis (arrow)* in a fine-needle pulmonary aspiration specimen from a dog with weight loss and respiratory distress. *(Courtesy of Dr. J.D. Hoskins, Louisiana State University.)*

ARTERIAL BLOOD GASES

Alveolar ventilation refers to ability of inspired air to enter and leave alveoli. *Ventilatory failure* refers to inadequate airflow into and out of alveoli and results in an inability to maintain carbon dioxide (CO_2) homeostasis in the body. *Respiratory failure* refers to failure of ventilation, perfusion, or diffusion.

Rare Indications • Measurement of arterial blood gases can be done in patients with airway or pulmonary parenchymal disease causing respiratory insufficiency or ventilatory failure of any cause.

Advantages • It quantitates the degree of respiratory impairment, evaluates compensatory changes, and monitors response to treatment.

Disadvantages • It does not allow the clinician to determine focal versus disseminated respiratory disease, to diagnose, or to prognosticate. In addition, it requires arterial puncture and prompt submission of the specimen.

Sample Procurement • See Chapter 6.

Analysis • An arterial blood sample is necessary for evaluation of the respiratory system. Use of venous blood gas analysis for determination of acid-base status is discussed in Chapter 6; venous O_2 tension (PvO_2) is discussed in the next section.

Normal Arterial Values • See Table 6-5.

Danger Values • Partial pressure of oxygen (PaO_2) less than 60 mm Hg; partial pressure of carbon dioxide ($PaCO_2$) greater than 70 mm Hg.

NOTE: Danger values depend on duration of the problem (chronicity allows compensatory mechanisms, enabling tolerance of greater abnormalities).

Artifacts • Improper sample storage before analysis can decrease the PaO_2 while failing to remove air bubbles can increase the PaO_2 (see Chapter 6).

Drug Treatment That May Alter Values • Excessive heparin decreases both pH and PaO_2, whereas citrate, oxalate, or ethylenediaminetetraacetic acid (EDTA) may decrease pH (see Chapter 6). Any drugs altering control of respiratory drive (e.g., anesthetic agents) may alter PaO_2.

Partial Pressure of Oxygen

Although measurement of PaO_2 helps evaluate the degree of respiratory dysfunction, it is only one factor affecting O_2 delivery to tissues. Other parameters, such as cardiac output, blood pressure, regional blood flow, position of the hemoglobin dissociation curve, and hemoglobin concentration, are also important (Table 11-4). PaO_2 in atmospheric air at sea level may range between 149 and 159 mm Hg (19.7% to 20.8%), depending on humidity.

TABLE 11-4. Causes of Hypoxemia in Dogs and Cats

Inadequate Oxygen in Inspired Air
High altitude
Failure of oxygen source during anesthesia
Alveolar Hypoventilation (associated with increased $PaCO_2$)
Depression/arrest respiratory center
Failure of muscles of respiration
Third-space disease (pneumothorax, pleural effusion, flail chest, diaphragmatic hernia)
Airway obstruction
Impaired Diffusion
Interstitial pulmonary disease (pneumonia, edema, neoplasia, embolism)
Airway disease
Vascular Shunting
Right-to-left cardiac shunts (tetralogy of Fallot, right-to-left shunting patent ductus arteriosus or ventricular septal defect or atrial septal defect)
Intrapulmonary arteriovenous shunts

The corresponding PaO_2 in alveolar air (104 mm Hg or 13.6%) is lower because of increased $PaCO_2$ and H_2O respiratory gases.

Causes of Hypoxemia • Causes include inadequate oxygen in inspired air, alveolar hypoventilation, impaired diffusion, ventilation/perfusion mismatching, and vascular shunting (see Table 11-4). Characterization and evaluation of hypoxemia and accompanying PCO_2 and HCO_3^- changes are discussed later under Diagnostic Evaluation of Blood Gases.

Partial Pressure of Carbon Dioxide

$PaCO_2$ in alveolar air at sea level is 40 mm Hg (5.3%), whereas that of atmospheric air is only 0.3 mm Hg (0.3%). An increase or decrease in $PaCO_2$ is caused by a decrease or increase in ventilation, respectively (Table 11-5). Because increased $PaCO_2$ decreases pH, the condition is also known as respiratory acidosis. The reverse occurs with a decrease in $PaCO_2$ (i.e., respiratory alkalosis).

Causes of Hypercapnia (Hypercarbia) • See Chapter 6.

Causes of Hypocapnia (Hypocarbia) • See Chapter 6.

Compensatory Responses to Alterations In Blood Gases • Decreased PaO_2 is associated with either increased or decreased $PaCO_2$. In dogs, predictable changes in HCO_3^- are associated with alterations in $PaCO_2$ (DiBartola and Aultran de Morais, 1992), and similar changes may occur in cats (see Table 6-2).

Diagnostic Evaluation of Blood Gases

First, the clinician should decide if the abnormality is significant. Minor elevations in PaO_2 are not an indication for additional evaluation other than characterization of the underlying disorder. In patients breathing an enriched O_2 mixture, a PaO_2 less than

TABLE 11-5. Assessment of Ventilation Based on Blood Gas Analysis

Decreased $PaCO_2$ (Hyperventilation):			
pH ↓-↓↓	pH normal	pH ↑	pH ↑↑
HCO_3^- ↓	HCO_3^- ↓	HCO_3^- ↓	HCO_3^- normal
Partially compensated metabolic acidosis	Compensated metabolic acidosis	Chronic hyperventilation (partially compensated respiratory alkalosis)	Acute hyperventilation (uncompensated respiratory alkalosis)
Normal $PaCO_2$:			
pH ↓-↓↓		pH ↑-↑↑	
HCO_3^- ↓		HCO_3^- ↑	
Uncompensated metabolic acidosis		Uncompensated metabolic alkalosis	
Increased $PaCO_2$ (Hypoventilation):			
pH ↓-↓↓	pH normal	pH ↑	
HCO_3^- normal	HCO_3^- ↑	HCO_3^- ↑	
Acute ventilatory failure (uncompensated respiratory acidosis)	Chronic ventilatory failure (compensated respiratory acidosis)	Partially compensated metabolic alkalosis	

Explanation of pH (dogs): pH normal: 7.36-7.44; pH ↑: 7.45-7.50; pH ↑↑: >7.50; pH ↓: 7.30-7.35; pH ↓↓: <7.30. Explanation of HCO_3^-. No attempt is made to quantitate HCO_3^- changes.

five times the inspired O_2 concentration is an indication for additional diagnostics. If the change is significant, one should first characterize the abnormality as a primary or secondary, compensated or uncompensated disorder and then seek to determine the underlying cause (see Table 11-5). A three-step process is recommended to characterize the abnormality. A brief overview of the three steps is given next. For additional information, readers are referred to DiBartola (1992b).

Step 1: Evaluation of the Ventilatory Status • The clinician should evaluate $PaCO_2$. Ventilation is classified as acceptable (i.e., normal $PaCO_2$), hyperventilation (i.e., decreased $PaCO_2$), or hypoventilation (i.e., increased $PaCO_2$). From Table 11-5, the abnormality can be assessed as respiratory or metabolic in origin. For additional information see Boxes 6-8 and 6-10 and the discussions of respiratory acidosis and respiratory alkalosis in Chapter 6.

Step 2: Assessment of the Hypoxemic State • Decreased PaO_2 confirms arterial hypoxemia and suggests tissue hypoxia (see Table 11-4).

Step 3: Assessment of the Tissue Oxygen State • Normal tissue oxygenation requires perfusion by adequately oxygenated blood. Therefore this step involves assessment of cardiac status, peripheral perfusion, and blood O_2 transport (see later).

PARTIAL PRESSURE OF OXYGEN IN VENOUS BLOOD

Occasional Indications • Measurement of PaO_2 helps evaluate adequacy of O_2 delivery to tissues and monitor cardiac output.

Analysis and Interpretation • If pulmonary edema is present, PaO_2 should be measured. If PaO_2 is greater than 65 mm Hg, partial pressure of oxygen in venous blood (PvO_2) reflects cardiac output (Kittleson, 1983). The sample should be taken from the jugular vein. Occlusion of the vein for longer than 5 to 10 seconds artifactually decreases PvO_2. Normal PvO_2 is greater than 40 mm Hg. Precautions for obtaining and storing the blood sample before analysis are the same as for arterial blood gases.

If PvO_2 is less than 30 mm Hg, O_2 delivery to the tissues is inadequate. Cardiac output, O_2 saturation, hemoglobin concentration, and peripheral arteriovenous shunts should be considered as causes of low PvO_2 and evaluated as described previously. In patients with cardiac disease, a PvO_2 cannot be correlated to a specific cardiac output, but increases or decreases in PvO_2 reflect improving or worsening cardiac output (respectively) relative to the initial value.

PULSE OXIMETRY

Occasional Indications • Pulse oximetry evaluates pulse rate and hemoglobin saturation (SaO_2). Measurement of SaO_2 is nearly as informative as PaO_2, because each is a measure of ability of the lungs to deliver oxygen to the blood. Pulse oximetry is valuable as an ongoing monitor to detect hypoxemia, especially during anesthesia.

Procedure • A pulse oximeter electrode is attached to the patient (e.g., tongue, lips, ear). Arterial blood gas measurements should be made periodically to verify accuracy of the pulse oximeter.

Interpretation • Reasonable pulmonary and cardiovascular function are required to achieve accurate measurements of pulse rate and hemoglobin saturation. Peripheral vasoconstriction may result in a poor reading of pulse rate but has value in identifying hypoxemia. Relationships between PaO_2 and SaO_2 with respect to hypoxemia are as follows: normal, SaO_2 greater than 90 and PaO_2 greater than 80; severe hypoxemia, SaO_2 less than 90 and PaO_2 less than 60; very severe hypoxemia, SaO_2 less than 75 and PaO_2 less than 40. SaO_2 would be expected to be normal with anemia but reduced with methemoglobulinemia or severe cardiopulmonary disease.

THORACOCENTESIS

Occasional Indications • Thoracocentesis is performed on patients with pleural effusions or mass lesions of the pleural cavity or mediastinum.

Advantage • It is relatively noninvasive.

Disadvantages • It has the potential to produce pneumothorax, hemothorax, or cardiac arrhythmias.

Procedure • Skin over the right 5th to 11th intercostal spaces (or elsewhere if effusion is localized) is clipped and prepared surgically. The needle of a butterfly-type catheter is inserted in the seventh to eighth intercostal space at approximately the level of the costochondral junction, and fluid is aspirated into a syringe. A three-way stopcock can be attached to the syringe if the procedure is to be both therapeutic and diagnostic. The method of fluid analysis is described in Chapter 10. Masses may also be aspirated for cytologic evaluation, ideally with fluoroscopic or ultrasonographic guidance. When fluid has been analyzed (see Chapter 10; Kuehn and Roudebush, 1991e), it may be useful to aspirate as much fluid as possible and radiograph (or reradiograph) the thorax to look for structures (e.g., mediastinal masses) not previously evident.

Interpretation • See Chapter 10.

THORACOSCOPY/THORACOTOMY

Rare Indications • Thoracotomy and thoracoscopy can be performed to look for a foreign body (e.g., grass awn) in patients with nocardiosis or actinomycosis or to look for infiltrative disease causing chronic or progressive pleural effusion. Thoracotomy is also used for lobectomy or biopsy (lung or masses) in patients with infiltrative disease that cannot be diagnosed with other tests. Thoracoscopy has the advantage of less morbidity and shorter postoperative recovery times compared with thoracotomy; however, thoracoscopy does not allow for as thorough an examination as does thoracotomy.

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12

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Immunologic and Plasma Protein Disorders

SERUM TOTAL PROTEIN AND ALBUMIN

Commonly Indicated • In most ill patients, but especially those with known or suspected anemia, edema, ascites, coagulopathies, diarrhea, weight loss, and hepatic or renal disease.

Advantages • Technically easy to perform.

Disadvantages • Additional testing is required to establish cause of altered protein concentrations.

Analysis • Total protein can be estimated in fluid, serum, or plasma (ethylenediaminetetraacetic acid [EDTA] or heparinized) by a refractometer that measures total solids. Total protein and albumin can be measured in serum, urine, or fluid by spectrophotometric or dry reagent methods. Serum globulin concentration is calculated by subtracting the serum albumin from the serum total protein. (See comments under Artifacts.)

Normal Values • Listed in Table 12-1. NOTE: Lower values are normal for perinates and very young animals. Values gradually increase until adulthood; the higher values are average normal values for adults. Both albumin and globulin tend to decline with advancing old age.

Danger Values • Albumin less than or equal to 1.0 g/dl can be associated with major fluid shifts in the body (may occur at higher serum albumin concentrations in patients with increased portal pressures). Patients with severe hypoalbuminemia are often deficient in antithrombin-III (AT III), thus also putting them at risk for pulmonary thromboembolism.

Artifacts • Falsely increased (refractometer): lipemia, hyperbilirubinemia, hemolysis, severe hyperglycemia, azotemia, hypernatremia, and hyperchloremia. *Important:* Certain methodologies that measure human albumin give falsely low values with canine albumin.

Drug Therapy That May Alter Protein Values • Hormonal changes generally have a slight effect on serum proteins, even though physical changes (e.g., body weight, muscle mass) may be marked. Hyperproteinemia may be caused by anabolic steroids, progesterone, insulin, and thyroid hormones in people.

TABLE 12-1. Normal Serum Total Protein and Albumin Values (g/dl)

	DOGS	CATS
Plasma total protein	6.0-7.8	6.0-7.5
Serum total protein	5.5-7.5	5.5-7.8
Serum albumin	2.5-4.0	2.5-4.0
Serum globulin	3.0-3.5	3.0-3.8

Prolonged, high-dose corticosteroid therapy can cause hyperproteinemia and hyperalbuminemia in normal dogs, but values return to normal within weeks after cessation of therapy (Moore et al, 1992). Hypoproteinemia may be due to estrogen; hypoalbuminemia may be due to anticonvulsants, acetaminophen, estrogens, and various antineoplastic agents in people.

Causes of Alteration in Plasma and Serum Protein • The serum total protein concentration is only important in that it allows calculation of serum globulin concentration.

Causes of Hyperalbuminemia • Dehydration and laboratory error are major causes.

Causes of Hypoalbuminemia • Concurrently evaluating serum globulin can sometimes help determine the cause of hypoalbuminemia. If both albumin and globulin values are decreased, hemorrhage, exudation from severe skin lesions, protein-losing enteropathy (PLE), and dilution are usually more important considerations (Table 12-2). Dilution usually causes mild decreases (albumin 2.1 to 2.4 g/dl), whereas PLE can cause moderate (1.5 to 2.0 g/dl) to severe (< 1.5 g/dl) hypoalbuminemia. PLE can be the result of primary intestinal disease or various causes of GI hemorrhage (e.g., mild-to-severe hypoalbuminemia was reported in approximately one third of over 40 dogs with Addison's disease [Langlais-Burgess, Lumsden, and Mackin, 1995], probably because of GI hemorrhage). Although both serum albumin and globulin are usually decreased in PLE, globulin concentration may be normal to increased in some cases.

Hypoalbuminemia plus normal to increased globulins suggests decreased albumin production, increased loss, or sequestration (see Tables 12-2 and 12-3). Increased albumin loss occurs in glomerular disease (and may be severe; see Chapter 7).

Albuminuria as the result of glomerulopathy is rarely associated with significant globulin loss. Decreased production is due to chronic hepatic insufficiency or hyperglobulinemia. The latter may cause mild hypoalbuminemia, whereas chronic hepatic insufficiency can cause moderate to severe decreases. In hyperglobulinemia, albumin synthesis may be decreased (i.e., "down regulation"). When inflammation is associated with hypoalbuminemia and hyperglobulinemia, albumin is sometimes called a *negative acute phase protein*. Inadequate protein intake (including poorly digestible protein), maldigestion, and malabsorption are rare causes of mild hypoalbuminemia; however, occasionally they accompany other conditions causing hepatic insufficiency or increased protein loss. Significant hypoalbuminemia (i.e., albumin < 2.1 g/dl) should never be attributed solely to decreased nutrition until hepatic insufficiency and protein-losing disorders have been eliminated by definitive tests (not just by history and physical examination). Decreased intake very rarely causes serum albumin concentrations less than 2.1 g/dl, except perhaps in very young animals.

TABLE 12-2. Causes of Hypoalbuminemia in Dogs and Cats

Decreased Production
Chronic hepatic insufficiency*
Inadequate protein intake†,‡
Maldigestion†
Malabsorption†
Hypergammaglobulinemia
Sequestration
Body cavity effusion
Vasculopathy
Increased Loss
Protein-losing nephropathy (PLN) because of glomerular disease*
Gastrointestinal: protein-losing enteropathy (PLE)*
Cutaneous
Whole blood loss
Dilution

*Most common and important causes of serum albumin ≤2.0 g/dl. Other causes rarely, if ever, cause serum albumin ≤2.0 g/dl.
†Of very doubtful importance as a sole cause of serum albumin ≤2.0 g/dl. Probably more important as a contributing factor when there is another problem that results in hypoalbuminemia.
‡Can be important in very young animals or animals fed diets that are extremely restricted in protein for prolonged periods.

TABLE 12-3. Causes of Hyperglobulinemia in Dogs and Cats

Polyclonal
Infections
- Bacterial*†
  - Brucellosis
  - Pyoderma
  - Bacterial endocarditis
- Viral
  - Feline infectious peritonitis (FIP)‡
  - Feline immunodeficiency virus (FIV)
  - Feline leukemia virus (FeLV)
- Fungal*†
  - Systemic fungal infections (e.g., blastomycosis, histoplasmosis, coccidioidomycosis)
- Rickettsial†‡
  - Ehrlichiosis
- Parasitic
  - Dirofilariasis*†
  - Demodicosis
  - Scabies
- Immune-mediated disease
  - Infections (immune complex)
    - Dirofilariasis*†
    - Feline cholangitis/cholangiohepatitis
    - Pyometra
  - Systemic lupus erythematosus (SLE), including glomerulonephritis, immune-mediated hemolytic anemia (IMHA) and thrombocytopenia (IMT), and polyarthritis*
  - IMHA, IMT (not because of SLE)*
  - Pemphigus complex, bullous pemphigoid*
  - Rheumatoid arthritis*
- Neoplasia†‡

Monoclonal
- Infection
  - Ehrlichiosis†‡
  - Leishmaniasis†‡
  - FIP *(rare)*
  - Idiopathic†‡
  - Benign monoclonal gammopathy
- Neoplasia†‡
  - Multiple myeloma‡
  - Macroglobulinemia
  - Lymphosarcoma
  - Extramedullary plasmacytoma *(rare)*
- Miscellaneous
  - Cutaneous amyloidosis
  - Plasmatic gastroenterocolitis*

*Mild (4 to 5 g/dl).
†Moderate (5 to 6 g/dl).
‡Severe (> 6 g/dl).
NOTE: Effect of age should be considered when assessing globulin values (see Causes of Hypoglobulinemia in text).

Sequestration may occur in pleural or peritoneal cavities or subcutaneous (SC) tissues. Thus patients with effusion caused by hypoalbuminemia may further lower their serum albumin concentration via sequestration. Alternatively, sequestration can be secondary to increased hydrostatic pressure (e.g., portal hypertension, right-sided cardiac failure). Immune-mediated or infectious vasculopathies (e.g., endotoxemia and bacteremia, ehrlichiosis, Rocky Mountain spotted fever [RMSF]) also allow loss from the vascular compartment. Hypoalbuminemia as the result of sequestration or vasculopathy is usually mild.

A diagnostic approach to hypoalbuminemic patients is outlined in Figure 12-1. A urinalysis (sometimes including urine protein:creatinine ratio; see Chapter 7) and measurement of serum bile acids (see Chapter 9) are indicated. Severe cutaneous exudative lesions may be diagnosed by physical examination, but the possibility of renal, hepatic, and alimentary disease should still be investigated. Hypercholesterolemia plus hypoalbuminemia suggests protein-losing nephropathy. Significant proteinuria indicates a diagnostic workup for protein-losing nephropathy (PLN) (see Chapter 7). Hypocholesterolemia plus hypoalbuminemia is suggestive of hepatic insufficiency or PLE. Hypoalbuminemia associated with hepatomegaly; microhepatia; neurologic signs; icterus; decreased blood urea nitrogen (BUN) with or without increased alanine aminotransferase (ALT), serum alkaline phosphatase (SAP), or both; or abnormal hepatic function test results (e.g., serum bile acids) requires a diagnostic workup for hepatic insufficiency (see Chapter 9). NOTE: ALT and SAP are normal in many patients with severe hepatic disease. A portosystemic shunt is more likely in young animals; however, congenital shunts can be diagnosed in animals more than 10 years old. Acquired hepatic disease is more common in adults and requires hepatic biopsy for diagnosis; however, some dogs less than 1 year old have severe, acquired hepatic disease. Hypoalbuminemia with normal liver function tests and absence of proteinuria or cutaneous lesions allows one to diagnose PLE by exclusion (see Chapter 9), even if feces are normal. If the patient has renal or hepatic disease and PLE is still a concern, then measurement of fecal alpha-1 protease inhibitor concentrations (Chapter 9) may allow diagnosis of PLE by inclusion. Intestinal biopsy may then provide a definitive diagnosis of which intestinal disease is causing PLE. Endoscopic biopsies are safer, but it is critical that excellent quality tissue samples be obtained (many endoscopically-obtained samples are nondiagnostic). Exploratory laparotomy is acceptable. If laparotomy is performed, hepatic biopsy should generally be performed along with intestinal biopsies. It is important to

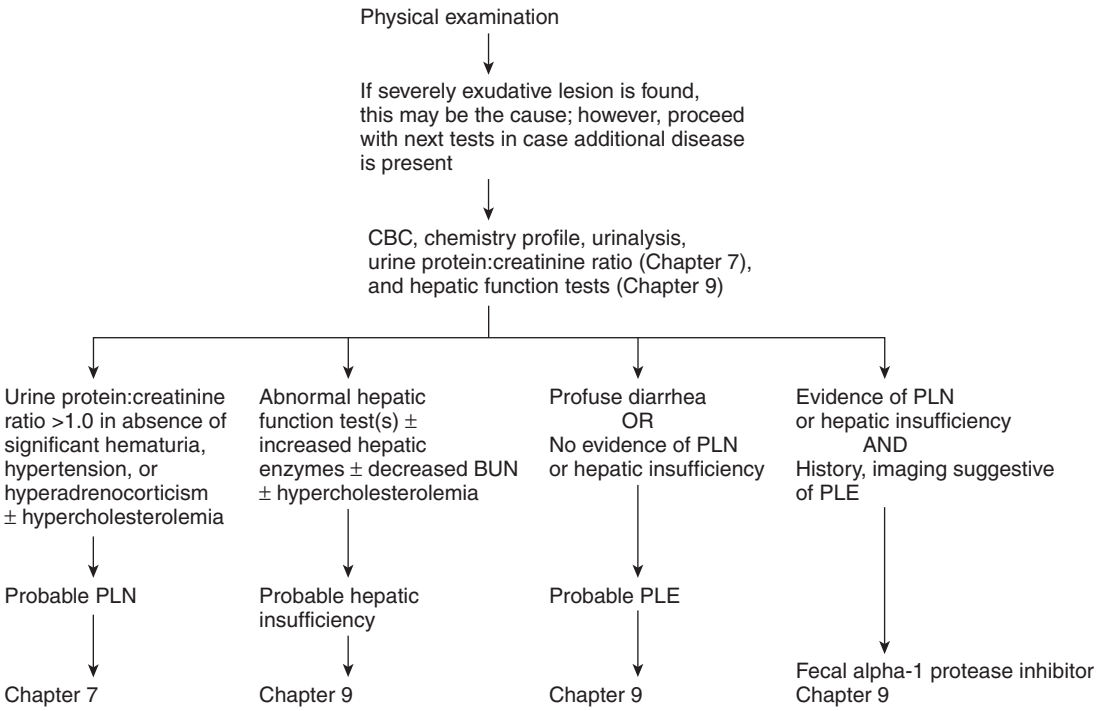

FIGURE 12-1. Diagnostic evaluation of hypoalbuminemia in dogs and cats when the serum albumin ≤2.0 g/dl. *BUN,* Blood urea nitrogen; *CBC,* complete blood count; *HI,* hepatic insufficiency; *PLE,* protein-losing enteropathy; *PLN,* protein-losing nephropathy.

obtain biopsy specimens at several sites along the small intestine, even when no apparent gross lesions are found.

Edematous SC fluid accumulations associated with hypoalbuminemia are usually transudates: PLN or PLE, chronic hepatic insufficiency, and immune-mediated or infectious vasculitis may be responsible. However, one should always sample fluid accumulations to be sure that they are in fact transudates, as opposed to unexpected modified transudates or exudates.

Causes of Altered Globulins • See following discussion of Protein Electrophoresis.

PROTEIN ELECTROPHORESIS

Occasionally Indicated • Protein electrophoresis is performed when hyperglobulinemia is not caused by hemoconcentration and either (1) the globulin concentration is high enough to make monoclonal gammopathy a reasonable possibility or (2) humoral immunodeficiencies are suspected. A pale-blue background on stained blood or bone marrow smears can represent increased plasma protein and may be an indication for protein electrophoresis.

Advantages • A useful screening test.

Disadvantages • Specific diagnosis is seldom obtained.

Although a specific diagnosis is seldom obtained from electrophoresis, electrophoretic patterns can be valuable when interpreted with clinical signs and other laboratory data. Electrophoresis is quantitative. Immunoelectrophoresis is qualitative, identifying specific proteins (e.g., immunoglobulins) but not detecting slight increases or decreases. Immunoelectrophoresis is the method of choice to detect urinary and serum Bence Jones protein, a monoclonal protein equivalent to immunoglobulin light chains which occasionally occurs in multiple myeloma and macroglobulinemia. A routine protein electrophoresis performed on a concentrated urine sample occasionally detects an isolated monoclonal peak in the urine (e.g., Bence Jones protein). Finding a urine electrophoresis pattern mimicking that of serum indicates

a glomerular lesion substantial enough to allow leakage of most serum proteins, including the serum monoclonal heavy chain peak; therefore, it is not evidence of Bence Jones light chains. Canine Bence Jones proteinuria is only rarely detected by heat precipitation. Positive results for Bence Jones proteins by an acid precipitation screening test should be confirmed by concentrated urine electrophoresis or immunoelectrophoresis because of the possibility of false-positive results.

Analysis • Serum or urine may be analyzed, and it may be refrigerated or frozen.

ELECTROPHORESIS

Analysis • The cellulose acetate technique is the method of choice. Interpretation of electrophoretograms is based on densitometric measurements of intensity of staining of protein bands on cellulose acetate strips. The serum separates into four fractions: (1) albumin, (2) alpha (α) globulins, (3) beta (β) globulins, and (4) gamma (γ) globulins. Canine and feline α-, β-, and γ-globulins are usually divided into two subfractions each: α_1, α_2; β_1, β_2; and γ_1, γ_2 (Table 12-4). Normal-appearing electrophoretograms from dogs and cats are presented in Figures 12-2 and 12-3.

Artifacts • Albumin concentration is usually underestimated by electrophoresis compared with a chemical determination. Therefore the albumin:globulin ratio (A:G) is usually higher by chemical determinations than by electrophoretic determination.

IMMUNOELECTROPHORESIS

Analysis • After electrophoresis in agar gel, polyclonal antiserum to specific proteins (including immunoglobulins) is added to a trough parallel with the separated serum proteins. The reagents are allowed to diffuse. To obtain quantitation of the individual immunoglobulins, radial immunodiffusion (RID), electroimmunodiffusion (i.e., rocket electrophoresis), or laser nephelometry is performed. These procedures, when available, can also be used to quantitate immunoglobulin subclasses.

Table 12-4. Normal Values (Mean ± 1 SD) for Serum Protein Electrophoresis in Dogs and Cats

	BREITSCHWERDT ET AL, 1987		KANEKO, 1980*	
DOGS	**MEAN**	**LIMITS**	**MEAN**	**LIMITS**
Total protein (g/dl)	6.84 ± 0.66	(6.0-7.6)	6.10 ± 0.52	(5.4-7.1)
Albumin†	3.20 ± 0.34	(2.72-3.67)	2.91 ± 0.11	(2.6-3.3)
α_1-globulin	0.33 ± 0.11	(0.25-0.60)	0.30 ± 0.03	(0.2-0.5)
α_2-globulin	1.13 ± 0.25	(0.72-1.40)	0.62 ± 0.21	(0.3-1.1)
β_1-globulin	0.74 ± 0.10	(0.63-0.89)	0.82 ± 0.23	(0.7-1.3)
β_2-globulin	0.79 ± 0.14	(0.59-0.96)	0.89 ± 0.33	(0.6-1.4)
γ_1-globulin	0.64 ± 0.15	(0.49-0.83)		
γ_1-globulin			0.80 ± 0.25	(0.5-1.3)
γ_2-globulin			0.70 ± 0.14	(0.4-0.9)
A:G ratio	0.89 ± 0.10	(0.79-1.02)	0.83 ± 0.16	(0.59-1.11)
CATS	**TURNWALD, BARTA, 1989**		**KANEKO, 1980***	
Total protein (g/dl)	7.66 ± 0.10	(7.3-7.8)	6.60 ± 0.70	(5.4-7.8)
Albumin†	3.41 ± 0.18	(2.82-4.18)	2.70 ± 0.17	(2.1-3.9)
α_1-globulin	0.47 ± 0.03	(0.30-0.64)	0.70 ± 0.02	(0.2-1.1)
α_2-globulin	0.55 ± 0.04	(0.41-0.68)	0.70 ± 0.02	(0.4-0.9)
β_1-globulin	0.91 ± 0.06	(0.77-1.25)	0.70 ± 0.03	(0.3-0.9)
β_2-globulin	0.40 ± 0.02	(0.35-0.47)	0.70 ± 0.02	(0.6-1.0)
γ_1-globulin	1.92 ± 0.12	(1.39-2.22)		
γ_1-globulin			1.60 ± 0.77	(0.30-2.50)
γ_2-globulin			1.70 ± 0.36	(1.40-1.90)
A:G ratio	0.80 ± 0.11	(0.63-1.15)	0.71 ± 0.20	(0.45-1.19)

*Numbers do not add up to the total protein values and A:G ratios as given in the table.
†Concentration of albumin is usually underestimated by electrophoresis compared with a chemical determination. Therefore the A:G ratio is usually higher by chemical determination than by electrophoretic determination.

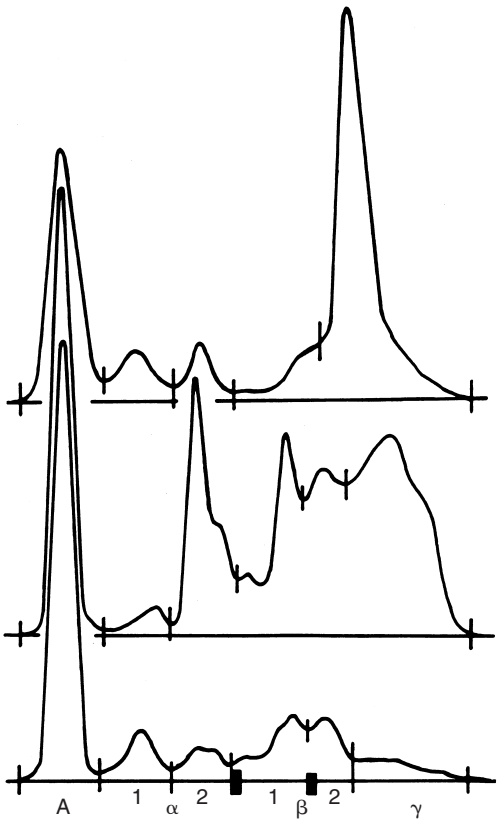

FIGURE 12-2. Electrophoretograms of different canine sera. *Top,* Monoclonal gammopathy (i.e., ehrlichiosis); *middle,* polyclonal gammopathy (i.e., blastomycosis, ehrlichiosis, dirofilariasis); *bottom,* normal.

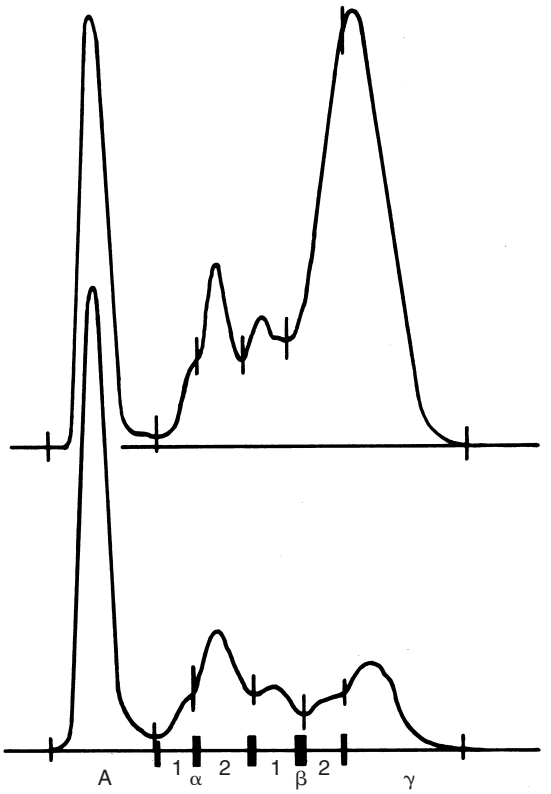

FIGURE 12-3. Electrophoretograms of feline sera. *Top,* Polyclonal gammopathy (i.e., feline infectious peritonitis [FIP]); *bottom,* normal.

Normal Values • Values vary among laboratories and the different techniques for quantitating individual immunoglobulins. Immunoglobulins migrate in the β_2 and γ regions of electrophoresis. Average concentrations of immunoglobulin classes are listed in Table 12-5.

NOTE: Normal values for puppies differ substantially from those for adults. Age-matched controls are recommended when submitting samples for immunoglobulin quantitation from young dogs because of great variation in reaching adult values among different antibody classes (Feldsburg, 1994). It is likely that breed-specific variations also occur.

Artifacts • Electrophoretic bands with high-intensity staining (e.g., albumin) are underestimated, and bands of low-intensity staining are overestimated. IgG migrates in β- and γ-globulin regions; therefore, IgG concentrations determined by RID are usually higher than the γ-globulin fraction determined by electrophoresis. This discrepancy increases when IgG hyperproduction occurs, such as in some myelomas, canine ehrlichiosis, feline infectious peritonitis [FIP], and other chronic infections.

Causes of Altered Electrophoretic Patterns • Diagnostic evaluation of patients with abnormal electrophoretograms is discussed under Causes of Hyperglobulinemia.

Causes of Hyperglobulinemia • Polyclonal hyperglobulinemias, also called *gammopathies*, have a broad-based peak encompassing β and γ regions and suggest persistent antigenic stimulation and inflammation (chronic bacterial, viral, fungal, protozoal, rickettsial, or parasitic disorders), neoplasia, or immune-mediated disease (see Table 12-3). The most common causes in dogs are cutaneous parasitism, pyoderma,

TABLE 12-5. Serum Immunoglobulin Concentrations in Dogs and Cats

	MEAN CONCENTRATION (mg/dl)				
	PUPPY (2 WEEKS)	PUPPY (2 MONTHS)	ADULT MONGREL DOG	ADULT PUREBREED DOG	ADULT CAT
IgA	Undetected	30	79	83	ND
IgG	56	143	1445	925	2400
IgM	73	118	45	156	ND

ND, Not done.
Data from Reynolds HY, Johnson JS (1970); Heddle RJ, Rowley D (1975); Schultz RD, Adams LS (1978); Reimann et al, 1986.

dirofilariasis, and ehrlichiosis, depending on geographic location (see Figure 12-2 and Table 12-3). Increases are commonly in the β and γ regions. In cats the most common cause of severe polyclonal gammopathy is FIP (see Figure 12-3). Increases in feline globulins are commonly in the γ region. A concurrent mild decrease in albumin synthesis may occur in patients with hyperglobulinemia, perhaps to maintain oncotic pressure or viscosity. In hypoalbuminemic states, globulin may increase secondarily.

Monoclonal hyperglobulinemias have a narrow-based electrophoretic peak (i.e., "spike") in the β or γ region, normally no wider than the albumin peak. Monoclonal immunoglobulin elevations are also called *paraproteins* or *M proteins* and are usually the result of lymphocyte and plasma cell neoplasias (e.g., multiple myeloma, macroglobulinemia, lymphosarcoma; see Table 12-3). Monoclonal or oligoclonal spikes are occasionally caused by infectious (e.g., ehrlichiosis; see Figure 12-2) or idiopathic disorders. Both multiple myeloma and ehrlichiosis can have monoclonal electrophoretic patterns and bone marrow plasmacytosis. In ehrlichiosis, however, the electrophoretic pattern is often a monoclonal or oligoclonal pattern superimposed on or arising within a broader-based globulin peak (see Figure 12-2, *top*). In such cases, examination of the stained electrophoretogram bands shows a clearly restricted monoclonal band with sharper edges within a paler, broader background band. In contrast, monoclonal spikes associated with neoplastic disorders are frequently accompanied by normal to decreased nonparaprotein globulin fractions, often reflecting impaired production of other immunoglobulins.

A suggested diagnostic approach to hyperglobulinemia is outlined in Figure 12-4. In dogs with hyperglobulinemia and severe pruritic dermatitis, diagnostic evaluation may involve only a physical examination to identify fleas and ticks or skin scrapings to detect mites. *Demodex canis* mites are usually detected easily, whereas *Sarcoptes scabiei* are often difficult to find. For canine heartworm disease, the enzyme-linked immunosorbent assay (ELISA) antigen test is the preferred screening procedure. In areas endemic for ehrlichiosis or RMSF, titers are indicated (see Chapter 15), particularly if the patient has anemia, thrombocytopenia, leukopenia (or a combination thereof).

Testing for other infectious disorders, such as canine brucellosis, blastomycosis, histoplasmosis, coccidioidomycosis, or feline cryptococcosis, is dictated by geographic location and other physical, laboratory, or radiographic abnormalities. The possibility of more than one cause of hyperglobulinemia should be considered. Gammopathies associated with immune-mediated disease and nonlymphocytic and plasmacytic neoplasia are usually mild and rarely require extensive evaluation of the gammopathy.

Feline corona virus (i.e., FIP) titers are generally not useful in cats with signs consistent with FIP. If an exudative effusion is present, fluid:serum γ-globulin ratios can be determined (see Chapter 15). Confirmatory testing for FIP can be performed on affected tissue biopsies, usually liver, using an immunohistochemical stain for FIP virus. Hyperglobulinemia can be the result of dirofilariasis in cats, but the disease is less common than in dogs. Hyperglobulinemia can also be present in chronic feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infections.

If joint pain, stiff gait, or increased joint fluid volume accompanies hyperglobulinemia, radiographs and joint fluid analysis (see Chapter 10) are indicated. Rheumatoid factor (RF) testing,

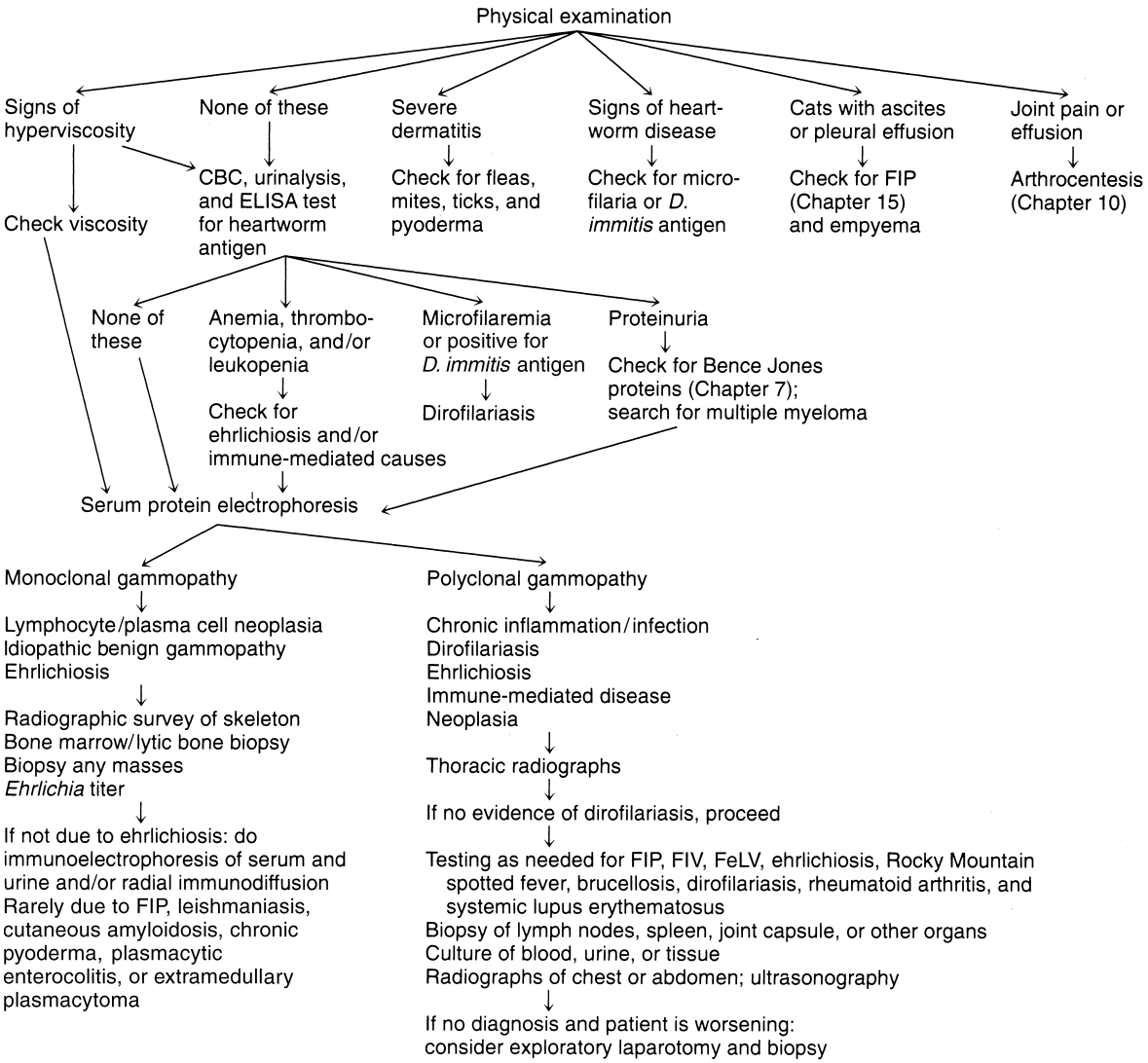

FIGURE 12-4. Diagnostic evaluation of moderate to severe hyperglobulinemia (globulin ≥5.0 g/dl) in dogs and cats. *CBC,* Complete blood count; *ELISA,* enzyme-linked immunosorbent assay; *FIP,* feline infectious peritonitis; *FeLV,* feline leukemia virus; *FIV,* feline immunodeficiency virus.

ANA testing, rickettsial titers, or a borreliosis titer (or a combination of these tests) may be helpful if the joint fluid is a nonseptic exudate.

If a diagnosis is not obtained at this stage, if a patient appears inappropriately ill, if the hyperglobulinemia seems excessive (e.g., globulin >5 g/dl), or if any signs consistent with hyperviscosity are present (see Serum Viscosity), one should differentiate monoclonal from polyclonal gammopathies by serum protein electrophoresis. If monoclonal gammopathy is detected in a patient with multiple myeloma or lymphosarcoma, immunoelectrophoresis or quantitation of immunoglobulins by RID (or rocket electrophoresis) identifies the class of immunoglobulin composing the paraprotein. These tests also detect nonparaprotein immunoglobulin deficiencies, which are common in patients with lymphocytic or plasmacytic neoplasias (see Causes of Hypoglobulinemia).

Polyclonal gammopathies may be caused by infectious, immune-mediated, or neoplastic disorders. If the cause of polyclonal gammopathy is unknown, thoracic and abdominal

imaging, various titers, and/or exploratory surgery plus biopsy may be indicated.

Monoclonal gammopathies are usually caused by lymphoproliferative (e.g., plasma cell) neoplasia. Evaluation of patients with monoclonal gammopathy may include skeletal radiographs, serum and urine immunoelectrophoresis, and bone marrow biopsy with cytologic and histologic evaluation. Diagnosis of multiple myeloma in dogs requires finding at least two of the following: lytic skeletal lesions, bone marrow plasmacytosis, Bence Jones proteinuria, or a monoclonal spike on serum protein electrophoresis. Skeletal lesions are uncommon in feline multiple myeloma. In areas endemic for ehrlichiosis, an *Ehrlichia canis* titer should be performed in dogs with a monoclonal gammopathy. Ehrlichiosis commonly causes proteinuria and bone marrow plasmacytosis, closely resembling multiple myeloma. FIP rarely causes monoclonal spikes. Serum viscosity can be measured.

Causes of Hypoglobulinemia • Newborn animals are physiologically hypogammaglobulinemic and have serum total protein concentrations 60% to 80% of adult values. Congenital combined or selective immunodeficiencies occur but are rarely diagnosed, probably because immunodeficient puppies or kittens rapidly succumb to infections. In dogs these infections are usually distemper or parvovirus, and they occur in the postnatal period after maternal immunity wanes. Immunodeficiency should be suspected when more than one pup in a properly cared for litter dies of infection in the first 2 to 6 months of life. Complete blood count (CBC), serum chemistry profile, serum protein electrophoresis, and immunoelectrophoresis are typical initial tests in such situations. Immunoglobulin quantitation by RID or rocket electrophoresis is recommended for confirmation and characterization of the type of immunoglobulin deficiency present.

Immunoglobulin quantitation techniques are more precise than qualitative methods (i.e., immunoelectrophoresis). Selective (single class or subclass) and partial immunoglobulin deficiencies may not be readily detectable via serum electrophoresis and immunoelectrophoresis; all immunoglobulin classes (and IgG subclasses when available) should be quantitated when a humoral immunodeficiency is suspected. Humoral immunodeficiency is usually detected by finding decreased IgG and IgA and normal to decreased IgM concentrations. Selective IgA deficiency and transient hypogammaglobulinemia occur in dogs; patients with selective IgA deficiency may show chronic problems involving mucosal immunity (e.g., antibiotic responsive enteropathy) or be asymptomatic. Serum electrophoresis in cases of selective or partial immunoglobulin deficiency may yield normal results or even show polyclonal elevations in globulins. Immunoelectrophoresis of normal canine serum may show a low or absent stainable IgA precipitation band because of IgA's relatively low concentration or the result of quality assurance problems involving antisera or technique. When selective IgA deficiency is suspected, the best diagnostic test is quantitative RID or rocket electrophoresis. IgA deficiency is sometimes accompanied by elevated concentrations of IgM or IgG.

The relationship between certain types of chronic inflammatory enteropathy (e.g., lymphocytic plasmacytic enteritis) and serum IgA deficiency is unclear at this time. It is likely that deficiency of local secretory IgA is not always reflected by serum IgA values. In addition, low concentrations of serum IgA have been associated with canine allergic and parasitic disorders, with return to normal after successful therapy. This finding suggests that those disease processes may lead to down regulation of IgA as an immunomodulation event, and low IgA probably has no causative role in the underlying chronic inflammatory bowel disease (Hill, Moriello, and DeBoer, 1995). Breed-specific normal ranges for immunoglobulins may provide a clearer picture of the role of selective IgA deficiency in chronic enteropathies.

The most common causes of hypoglobulinemia are external blood loss and PLE. Less common causes are PLN and hepatic insufficiency (see Chapters 7 and 9, respectively). In patients with paraproteinemias (e.g., multiple myeloma, macroglobulinemia, lymphosarcoma), remaining immunoglobulins are usually depressed. A complement measurement and evaluations of lymphocyte and phagocyte functions may also be indicated for patients with suspected congenital immunodeficiency (see Testing for Cellular Immunity).

SERUM VISCOSITY

Rarely Indicated • Monoclonal gammopathy, hyperglobulinemic patients with signs of hyperviscosity (i.e., poor tissue perfusion, dilated retinal vessels, retinal hemorrhage or

retinal detachment, renal disease, central nervous system (CNS) dysfunction, bleeding problems), and monitoring of treatment of diseases causing hyperviscosity. Hyperviscosity may be suspected based on finding high serum protein (usually >10 g/dl) or on physical characteristics of serum (i.e., viscous). Polycythemia should be included as a cause of increased blood viscosity to be eliminated in patients showing clinical signs of hyperviscosity.

Advantages • Simple and diagnostically significant.

Analysis • Measured in serum with an Ostwald viscosimeter or a 0.1 ml capillary pipette. Time for a given volume of serum to flow from the pipette is compared with that for the same volume of water.

Normal Values • Relative viscosity, 1.4 to 1.8. (Relative viscosity = flow time of serum [seconds] divided by flow time of water [seconds].)

Artifacts • Volume depletion causing increased serum protein concentration may increase serum viscosity but is unlikely to be clinically significant. Markedly increased blood viscosity can interfere with tests using flow through devices (e.g., hematology autoanalyzers).

Drug Therapy That May Alter Serum Viscosity • Any drug causing volume depletion can increase serum viscosity.

Causes of Serum Hyperviscosity • A relative viscosity greater than or equal to 4 is abnormal in people and probably abnormal for dogs. Because of the relatively large size of IgM, it has the greatest potential to cause hyperviscosity. IgA (which can exist as a polymer or dimer) and very high concentrations of IgG can also cause hyperviscosity. Clinically significant serum hyperviscosity is almost invariably caused by lymphocyte and plasma cell neoplastic disorders (e.g., multiple myeloma, macroglobulinemia, lymphosarcoma; see Table 12-3). Hyperviscosity syndrome rarely occurs in monoclonal gammopathy caused by ehrlichiosis.

Diagnostic approach is described in Figure 12-4 under Monoclonal Gammopathy. Because lymphosarcoma and plasma cell myeloma are the major causes of serum hyperviscosity, aspiration of bone marrow, involved lymph nodes, masses, or other abnormal lymphoreticular organs is indicated. If results are equivocal, biopsy and histopathologic evaluation of bone marrow or involved tissues are indicated. Cytologic or histologic evaluation of spleen and liver occasionally helps diagnose lymphocytic or plasmacytic neoplasia when samples from lymph nodes, bone marrow, or solid masses are not diagnostic.

CRYOPRECIPITATION

Cryoglobulins are usually monoclonal or complexed immunoglobulins that reversibly precipitate or gel at low temperatures but dissolve when heated. Cryoglobulins are rarely found in canine multiple myeloma and macroglobulinemia.

Rarely Indicated • Paraproteins that precipitate or gel when blood or serum is refrigerated at 4° C.

NOTE: Cryoglobulins are not cold agglutinins (i.e., antibodies binding antigen reversibly at temperatures <37° C).

Advantage • Detection of cryoglobulins is important, because failing to detect them may cause false-negative tests for hyperglobulinemia or monoclonal paraproteinemia in patients with clinical or laboratory evidence of hyperviscosity.

Analysis • The clinician should contact a reference lab about assaying for cryoglobulins.

Causes of Cryoglobulinemia • Macroglobulinemia or multiple myeloma of IgM and IgA classes may cause canine cryoglobulinemia. Finding cryoglobulinemia indicates diagnostic evaluation for lymphocyte and plasma cell neoplasia as described for monoclonal gammopathies.

ANTINUCLEAR ANTIBODY

Occasionally Indicated • Abnormalities suggestive of systemic lupus erythematosus (SLE), such as symmetric dermatitis principally distributed on the head and mucous membranes, hemolytic anemia, thrombocytopenia, nonseptic polyarthritis, myositis, proteinuria, or fever of unknown origin. Less common are neuromuscular, cardiac,

or pulmonary abnormalities. The test can be used to monitor patients being treated for SLE.

Advantages • Simple and indicative of immune-mediated disease when positive with a relatively high titer in conjunction with compatible clinical abnormalities.

Disadvantages • Not a disease-specific test (i.e., many diseases besides SLE may be associated with a low titer; Table 12-6).

Analysis • Measured in serum by indirect immunofluorescence testing (IIT). The result should be reported as the highest dilution of a patient's serum causing definite staining of nuclei. Several patterns of nuclear fluorescence are recognized: homogeneous (diffuse), rim (peripheral), speckled (fine or large speckles), and nucleolar.

Normal Values • Values vary among laboratories owing to different substrates, controls, and procedures used. Accuracy requires procedural consistency and experience. Fetal and newborn sera do not stain nuclei. Most veterinary laboratories use tissue culture monolayers of human epithelial-2 (HEp-2) cells as substrate, which allows improved discernment of fluorescent patterns of staining and more standardized procedural consistency. When this test method was used in a comparative study involving 112 canine serum samples, a significant positive titer was established at a screening dilution of 1:25 for greater than 95% specificity, whereas a minimum significant ANA titer of 1:100 was established as the corresponding significant titer using rat liver sections, which identified the identical group of ANA-positive dogs at the same specificity of greater than 95% (Hansson, Turnwald-Wigh, and Karlsson-Parra, 1996).

Drug Therapy That May Alter Antinuclear Antibody Titer • Anything decreasing antibody synthesis (e.g., cytotoxic drugs, chronic or high-dose corticosteroid therapy) can decrease the titer. Positive ANA titers have been attributed to treatment with griseofulvin, hydralazine, procainamide, sulfonamides, and tetracyclines. Positive ANA titers can occur in cats treated with propylthiouracil or methimazole (Peterson, Kintzer, and Hurvitz, 1988). Some of these cats develop drug-induced, immune-mediated hemolytic anemia (IMHA) and immune-mediated thrombocytopenia (IMT).

Artifacts • Improper reagent preparation, storage, or application; inadequate controls.

Causes of Increased Antinuclear Antibody Titer • A positive titer may occur in a number of infectious, inflammatory, and neoplastic disorders. A partial list of diseases (in addition to SLE, in which a positive ANA titer can be found) is given in Table 12-6. Normal dogs and cats can also have detectable ANA; however, these tend to be low titers. Positive titers obtained in disorders other than SLE are generally not markedly elevated; therefore, it is important to consider values established by the laboratory for low, moderate, and high titers. Equally important is consideration of other clinical and clinicopathologic changes consistent with a diagnosis of SLE.

Suggested criteria for diagnosis of canine and feline SLE are a positive ANA titer plus one or more of the following: skin or oral cavity lesions with histopathologic and immunopathologic changes consistent with SLE, polyarthritis, Coombs'-positive hemolytic anemia, idiopathic or IMT, PLN, myositis, or (particularly in cats) neurologic disturbances.

A positive ANA titer is the most important criterion for diagnosis of SLE, providing

TABLE 12-6. Causes of Increases Antinuclear Antibody Titer in Dogs and Cats

Systemic Lupus Erythematosus (SLE) (titer most consistently elevated in this disease)*
Skin Disorders
Pemphigus erythematosus (seldom pemphigus vulgaris)†
Discoid lupus
Generalized demodicosis
Fleabite hypersensitivity
Plasma cell pododermatitis
Hematologic Disorders
Immune-mediated hemolytic anemia (IMHA)
Immune-mediated thrombocytopenia (IMT)
Cardiopulmonary Disorders
Bacterial endocarditis
Dirofilariasis
Other Disorders
Cholangiohepatitis
Feline leukemia virus (FeLV)
Feline infectious peritonitis (FIP)
Rheumatoid arthritis
Lymphocytic thyroiditis
Various neoplasms
Ulcerative autoimmune stomatitis†

*Moderate to high titers.
†Moderate titers.
NOTE: ANA titer, if positive, in disorders without * or † is likely to be low.

established clinical criteria are met and exclusionary diagnoses are not made (e.g., FeLV or FIP infection, cholangiohepatitis, rickettsial or systemic parasitic diseases). No well-established patterns of ANA fluorescence in cats and dogs distinguish SLE from other (i.e., non-SLE) immune-mediated diseases or other conditions associated with positive ANA titers (see Table 12-6). Although ANA titers in SLE are frequently higher than in other disorders, there can be some overlap. Magnitude of titer does not parallel severity of disease. Periodic ANA titers may be useful in monitoring a lupus patient's response to therapy.

A positive ANA titer can be an indication for performing additional tests to distinguish SLE from non-SLE disease: dermatitis favoring mucocutaneous junctions is an indication for biopsy, histopathologic examination, and immunohistochemical or direct immunofluorescent testing (DIT; see Immunostaining of Tissues); hemolytic anemia is an indication for an antiglobulin (i.e., Coombs') test (see Chapter 3) and tests for hemoparasites; swollen, painful joints are an indication for arthrocentesis, fluid analysis (see Chapter 10), and RF test (see later discussion).

LUPUS ERYTHEMATOSUS TEST

Occasionally Indicated • Suspected SLE (see Antinuclear Antibody).

Advantages • Specific; does not require species-specific reagents, therefore more widely available.

Disdvantages • Time-consuming and much less sensitive than the ANA test; requires very fresh blood sample.

Analysis • Depending on the laboratory, heparinized or clotted blood is used. Formation of LE-cells *in vivo* is rarely demonstrated in routinely stained bone marrow smears or in joint fluid from patients with polyarthritis (Color Plate 5A), but it is highly suggestive of SLE when present. A laboratory experienced in performing and interpreting LE cells is necessary.

Artifacts • LE cells must be differentiated from "tart" cells, which are neutrophils that have phagocytosed intact nuclei. The LE cell test is complement dependent, and low concentrations of complement, excessive heparin, or failure to use freshly drawn blood may cause false-negative results.

Drug Therapy Causing False-Negative Results • Steroid therapy alters test results. The LE cell test is more sensitive to effects of steroids than is an ANA titer.

Causes of Positive Lupus Erythematosus Cell Preparations • Ideally a minimum of three to four LE cells on a slide is necessary for a diagnosis of SLE. The test should be performed at least three times before results are considered negative. The test is specific for SLE but insensitive. Negative results are common in SLE patients that are ANA positive. Such patients may lack the particular autoantibodies to histone-DNA involved in LE cell formation but have other types of ANA detectable by immunofluorescence. Positive test results may rarely be obtained in other diseases (e.g., osteochondritis dissecans, nonimmunologic joint disease, neoplasia, disseminated intravascular coagulation). If a positive LE cell preparation is obtained, an ANA titer and tests to obtain other evidence of SLE are indicated (as described earlier under Antinuclear Antibody). An ANA titer is the preferred screening test for SLE.

ANTIGLOBULIN (COOMBS') TEST

See Chapter 3.

TESTS FOR IMMUNE-MEDIATED THROMBOCYTOPENIA (IMT)

Dogs and cats showing marked thrombocytopenia (<50,000/µl) may have IMT. This is typically a diagnosis of exclusion because no widely available test allows a definitive diagnosis of IMT. Measurement of platelet-associated antibodies and assay for antimegakaryocyte antibodies have been performed, but as of this writing, neither has been proven to have the desired sensitivity or specificity in clinical practice. A recently reported technique seems promising (Scott et al, 2002).

RHEUMATOID FACTOR TEST

Occasionally Indicated • Dogs, particularly small breeds, suspected of having

rheumatoid arthritis (RA) because of lameness, heat, swelling, or pain of multiple joints, particularly peripheral joints. Crepitation and joint laxity may be detected in chronic cases. Nonspecific signs include anorexia, fever, depression, and reluctance to move and may precede clinically or radiographically detectable evidence of joint disease.

Disadvantages • Insensitive (i.e., many false-negative results) and not highly specific for RA in dogs because of relatively low titers.

Analysis • Serum is submitted refrigerated but unfrozen. The Rose-Waaler test is the recommended test for canine RF (autoantibody reacting against a patient's own IgG). Canine RFs are predominantly IgG but may also be mixed complexes of IgG, IgA, and IgM. Factors are detected by incubating rabbit IgG-sensitized sheep red blood cells (RBCs) with serial dilutions of the patient's serum (the rabbit Rose-Waaler test). If RF is present, agglutination occurs. Because canine sera often contain naturally occurring antibodies to sheep RBCs, a control must be used with unsensitized sheep RBCs. If agglutination to an equal or higher dilution appears in the control, natural antibodies must be absorbed from the sera before testing for RF. Latex agglutination tests for canine RF are not recommended because results are poorly reproducible and lack specificity. Latex agglutination titers may differ substantially from Rose-Waaler titers. Both tests are available through human laboratories; proper test controls are necessary.

Results • For the Rose-Waaler test in normal animals, the differential titer (difference between titers at which agglutination of unsensitized versus sensitized sheep RBCs occurs) should be less than eight (Barta, 1993). A titer against sensitized RBCs not corrected for the actual titer of natural antibodies to sheep RBCs can be misleading because some normal animals have higher titers of naturally occurring antibodies to sheep RBCs. Some laboratories perform the Rose-Waaler test by preabsorbing all test sera with sheep RBCs. In this case a titer of less than 16 is expected in normal dogs.

Artifacts • Serum submitted for RF testing should not be frozen, because RF activity (especially IgM) may be destroyed, causing a false-negative result. Canine RF tends to self-associate, forming multimeric complexes that significantly lower the detectable titer. Wide, sometimes negative, fluctuations in RF titer occur over time; these fluctuations do not appear to correlate with disease severity. False-positive results may occur in the Rose-Waaler test if a patient's serum has antibodies against sheep RBCs and appropriate controls or absorption of these antibodies is not performed.

Causes of an Increased Rheumatoid Factor Titer • Because RF is an antibody against Fc fragments of immunoglobulin molecules that become exposed only after antibody binds to antigen, any disease with long-standing immune complexes can eventually induce RF formation. In the Rose-Waaler test, a differential titer of greater than or equal to 1:8 is positive for RF (Barta, 1993). Between 40% and 75% of dogs with RA have a positive RF test result. Hence, a negative result does not eliminate RF. The RF test is rarely positive in normal dogs and occasionally positive in some patients with SLE, because RF may be a part of the SLE complex. Incidence of RF in other arthropathies and systemic diseases has not been adequately studied via the Rose-Waaler method, but other methods have shown RF (in titers comparable with those of patients with RA) in these arthropathies (Carter et al, 1989). Therefore a positive RF test result should never be the sole criterion for a diagnosis of canine RA.

RA is a progressive, erosive, immune-mediated polyarthritis that must be differentiated from other types of joint diseases, preferably before joint destruction occurs. Unfortunately no test for canine RF is highly reliable in making this distinction. Other routine tests indicated in making the diagnosis include joint radiographs and synovial fluid analysis. Septic polyarthritis often causes erosive lesions, especially involving larger joints, plus evidence of sepsis on routine blood and synovial fluid examinations (see Chapter 10), including culture. In contrast, RA typically causes erosive lesions of smaller peripheral joints before progressing to larger joints. Radiographic lesions may be lacking or inconclusive early in the course of RA. Furthermore, no distinguishing cytologic features can reliably differentiate among RA, SLE, and other types of immune-mediated joint disease that have similar joint fluid cytology. Histopathologic examination of synovium from affected

joints is the most reliable means of diagnosing canine RA.

Histopathologic examination of synovium allows an early diagnosis and therapy in patients lacking classic radiographic changes. An ANA titer helps distinguish SLE from RA; but it is occasionally positive for RA. Finding both types of autoantibodies may represent the rare, combined occurrence of both SLE and RA (a so-called overlap syndrome) or merely the appearance of multiple autoantibodies in a patient with RA or SLE. In either case, clinical criteria are required for diagnosis.

IMMUNOSTAINING OF TISSUES

Immunofluorescent testing of tissues uses a fluorescent dye conjugated with an antibody (i.e., fluorescein isothiocyanate [FITC] reagent) that detects antigen, immunoglobulin, or complement. Several techniques are used for immunohistochemical staining involving enzyme-conjugated antibody in a chromogenic reaction system. The testing may be direct or indirect. In the direct test, tissues are assayed for deposits of antigen, immunoglobulins, or complement.

Occasionally Indicated • Suspected autoimmune skin disease or (rarely) diseases of internal organs (e.g., kidney) that may have an immunologic basis. In veterinary medicine, the test is used mainly on biopsy specimens of erosive or vesiculobullous cutaneous lesions.

Advantages • A definitive diagnosis can occasionally be made; formalin-fixed tissue can be used for some immunohistochemical procedures, allowing routine histopathology and immunostaining on sections from the same biopsy.

Disadvantages • Extreme care must be taken in tissue selection and preservation. The test result is markedly influenced by corticosteroids; some immunofluorescence procedures require a special fixative and transport medium or fresh, snap-frozen tissue.

Sample Preparation • Requirements for handling and submitting biopsied tissues vary according to the procedure and reagents used, so the laboratory should be contacted. In cutaneous diseases it is imperative that early primary lesions (i.e., vesicles, bullae, pustules) and a margin of uninvolved skin be obtained. Multiple specimens are recommended. Obtaining primary lesions may necessitate checking a patient several times daily to identify a suitable, newly erupting lesion. Ulcerated, crusted, or scarred lesions are worthless. Ideally, biopsy specimens should not be obtained from the planum nasale or foot pads if other primary lesions are available because positive staining occurs at the basement membrane of these sites in many normal dogs. Biopsy specimens for routine histopathology should be taken from the same, or at least a similar, lesion. Larger samples can be bisected to provide comparable material for both tests. The biopsy specimen should not remain attached to the wall of the fixative container because this may cause inadequate preservation and false-negative results.

Analysis • Tissue sections are prepared and tissue incubated with labeled antibodies to IgG, IgM, IgA, or C3 and examined by fluorescent microscopy for immunofluorescent assays or routine microscopy for immunohistochemical assays.

Normal Values • Healthy epidermis has no detectable deposits of immunoglobulins in the intercellular spaces or at the basement membrane. Nonspecific fluorescence of the stratum corneum should not be mistaken for deposits of immunoglobulins. Appropriate controls are necessary to detect nonspecific background staining.

Artifacts • Biopsy of inappropriate lesions produces false-negative results. Cutaneous bacterial infections may cause immunoglobulin deposits in pustules, which should not be mistaken for autoimmune disease.

Drug Therapy That May Affect Results • Corticosteroids and immunosuppressive drugs (e.g., cyclophosphamide) may cause negative results. If an animal is receiving short-acting corticosteroids, the drug should be withdrawn for at least 3 weeks before biopsy. A longer period (1 to 2 months) may be necessary if long-acting injectable corticosteroids have been used.

Causes of Positive Immunostaining • Immunostaining results must be interpreted in light of clinical and histopathologic findings. Immunoglobulin deposits can occur in

other skin disorders, including mycosis fungoides, pyodermas, and acariasis. In these disorders, staining is usually patchy, focal, and present in pustules rather than in the adjacent tissues.

INDIRECT IMMUNOFLUORESCENT TESTING (IIT)

In the indirect test, serum is assayed for circulating autoantibodies to a specific tissue component, such as cutaneous basement membrane, intercellular cement substance, or renal glomerular basement membrane. The ANA test is another example of IIT discussed previously.

Rarely Indicated • As an adjunct test for suspected pemphigus, bullous pemphigoid, or immune-mediated glomerulonephritis.

Advantages • Noninvasive method to detect circulating autoantibodies to tissue, especially when biopsy of these organs is problematic or nondiagnostic.

Disadvantages • Significantly less sensitive than direct immunostaining, because a relatively large amount of antibody must be present to be detected. This test so rarely gives positive results in confirmed cases of autoimmune skin disease or immune-mediated glomerulonephritis that it is *not recommended*.

TESTING FOR CELLULAR IMMUNITY

Routinely available tests are used to screen suspected cases of immunodeficiency. These tests include serial CBC examinations (total lymphocyte and neutrophil numbers), serum immunoglobulins (see Causes of Hypoglobulinemia), and antibody titers to common viral vaccines such as distemper or parvovirus. Sustained lymphopenias (<1000/µl) are noted in some cases of severe combined immunodeficiency, and marked neutrophilias (>50,000/µl) are often observed in neutrophil function or chemotactic disorders (see Chapter 4). Routine histologic evaluation and immunophenotypic staining of lymph node tissue taken at biopsy are occasionally useful in pursuing a diagnosis of congenital or acquired immunodeficiency. Possible abnormal findings include depletion or altered distribution of B-lymphocyte or T-lymphocyte populations within the nodal architecture. Tests for cellular immunity include lymphocyte blastogenesis (i.e., transformation), assays for cell-mediated cytotoxicity, enumeration of T-lymphocyte and B-lymphocyte subpopulations, neutrophil function, and evaluation of leukocytic and erythrocytic histocompatibility antigens. If cellular immunity needs to be evaluated, the veterinarian should contact a college of veterinary medicine or large diagnostic laboratory to determine availability.

EVALUATION OF PHAGOCYTES

Clinically significant phagocytic function disorders tend to occur in purebreds (e.g., Irish setters, Weimaraners) or closely related crossbreds as a congenital or inherited defect. These animals show chronic or recurrent bacterial infections from a young age, along with markedly elevated neutrophil counts and general unthriftiness. Tests include phagocytic assays, bactericidal assays, chemotactic response, and testing for cell surface markers such as leukocyte adhesion molecules LFA-1 and Mo-1. The veterinarian should contact a college of veterinary medicine to determine availability.

ENUMERATION OF PROPORTIONS OF T AND B LYMPHOCYTES

Occasional Indications • Poor response to vaccinations, chronic unresponsive infections, persistently elevated or decreased lymphocyte counts, distinguishing between neoplastic and reactive lymphoproliferation by evaluating clonality.

Analysis • Studies are usually performed on peripheral blood lymphocytes but can also be performed on lymph node tissue obtained by biopsy. The clinician should consult individual laboratories for sample specification.

Causes of Altered Proportions of T and B Cells • Inherited defects of the immune system, lymphosarcoma, lymphocytic leukemia, and some viral infections (especially retroviruses such as FeLV, FIV, and other viruses infecting lymphoid cells) change the proportions of T and B cells.

EVALUATION OF LYMPHOCYTE FUNCTIONS IN VITRO

Occasional Indications • Poor vaccination response or recurrent infections. The lymphocyte transformation test is the most commonly used test for assessing functional capability of lymphocytes.

Disadvantages • The test is not readily available and is only a simulation of an *in vivo* situation; therefore, the results should be interpreted as an approximation only.

Analysis • Isolated lymphocytes are exposed to various substances that cause their activation and division. The proliferation response of stimulated cells is quantitated by their increased uptake of radionucleotide compared with control cells from healthy animals of the same species and similar age. These tests can detect intrinsic lymphocyte function defects and serum suppressive factors, which can be found in many acquired disease states. If evaluation of lymphocyte function testing *in vitro* is contemplated, the reader should contact the local or regional veterinary school immunology laboratory for additional information on availability of testing.

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13

Cheri A. Johnson

Reproductive Disorders

GENERAL DIAGNOSTIC APPROACH

Clinicopathologic testing of the reproductive system is often indicated in normal animals (e.g., routine prepurchase and prebreeding examinations, optimizing conception rates and litter size, aiding pregnancy management, assessing semen quality before freezing) and abnormal animals (e.g., infertility, problems with pregnancy and parturition, discharges from external genitalia, abnormal sexual behavior, abnormal conformation of the genitalia). The most important diagnostic procedures are history, and physical examination, and then vaginal cytology in females and semen evaluation in males.

Reproductive history should include methods used to screen for infectious diseases affecting reproduction (e.g., *Brucella canis* in dogs and viral rhinotracheitis in cats) (see Chapter 15), medications known to affect reproductive function (e.g., glucocorticoids, thyroid hormones, gonadotropins, estrogens, progestins, androgens, prostaglandins), familial association with infertility, and stressful activities (e.g., travel, being shown, hard work [racing, hunting, training]).

For females, the history of estrous cycles should include the dates of the onset of each cycle, physical and behavioral characteristics of the female during each cycle, duration of each estrous period, and breeding dates and method of insemination (natural or artificial). The stud's reproductive performance with other bitches before and after breeding the bitch in question is important. Dates and methods of pregnancy examination and any signs of abortion should be noted. If pregnancy occurred, was parturition normal? What were gestation length and litter size?

For males, history should include libido, mating ability, number of females bred, conception rate, breeding methods (i.e., natural or artificial insemination, frequency of use, how insemination dates are chosen), and the

reproductive performance of bitches before and after being bred to the male in question.

Physical examination of the male reproductive tract includes inspection of preputial and scrotal skin and of the penis. Both testes, epididymides, and spermatic cords should be palpated. The prostate is palpated both rectally and transabdominally. Physical examination of the female includes inspection of the vulva and vestibule. The uterus is palpated transabdominally. The posterior vagina is palpated with a gloved finger (depending on size of patient); rectal palpation may also be helpful.

VAGINAL CYTOLOGY

Common Indications • Vaginal cytology is used to monitor the stages of the estrous cycle, to determine breeding and whelping management, to investigate abnormal estrous cycles, to investigate "mismating," and to determine the nature of vulvar discharges and other reproductive disorders. Vaginal cytology does not identify ovulation.

Technique • A saline-moistened cotton-tipped swab is inserted into the vagina, or saline is flushed into and aspirated from the vagina with a pipette. One should avoid the clitoris, the clitoral fossa, and the skin when obtaining the sample, because these areas contain keratinized cells that confuse the interpretation. Next, the swabs are gently rolled onto slides, or drops of the saline aspirate are placed on slides. Slides are then fixed and stained (new methylene blue [NMB], Wright's-Giemsa, Wright's stain, trichrome stain, and modified Wright's-Giemsa [Diff-Quick] are commonly used, but many others are acceptable).

NMB may be used on wet or air-dried preparations. A drop of stain is placed on the cells on the slide, and a coverslip is used. Slides can be read immediately. Disadvantages of NMB staining are (1) red blood cells (RBCs) are not stained and (2) slides must be read immediately. Wright's, Wright's-Giemsa, and trichrome stains require several different staining solutions, and instructions must be followed carefully. This makes them cumbersome; however, all cell types are stained, and slides can be stored. Diff-Quik stain is a reasonable compromise. Slides are fixed in methanol and then dipped in the two Diff-Quik solutions. Vaginal epithelial cells may stain more slowly than blood cells, so staining time may need to be increased by a few "dips" more than the manufacturer's recommendations. Slides can be stored for days without a coverslip, although a coverslip prolongs storage time. A minimum of 100 epithelial cells are examined and tabulated.

Interpretation • The cell types of the vaginal epithelium have been described (Olson et al, 1984a). From the lamina propria to the epithelial surface, in order of increasing maturity, they are basal, parabasal, intermediate, superficial-intermediate, and superficial cells. The basal cells are on the basement membrane and are not exfoliated. Parabasal cells are small and round, with a large nuclear: cytoplasmic ratio. Intermediate cells are round, about twice the size of parabasal cells, and have a similar nucleus. Superficial-intermediate cells are larger, have angular borders, and are often folded. The nucleus is still large but beginning to show karyolysis. The superficial cells are angular, thin, and folded. The nucleus is pyknotic or takes up no stain if very karyolytic (Figure 13-1).

Estrogen causes proliferation, thickening, and maturation of the vaginal epithelium. Therefore vaginal cytology can be used to monitor the estrous cycle, especially the follicular phase of proestrus and estrus when the ovarian follicles are producing estrogen (Table 13-1).

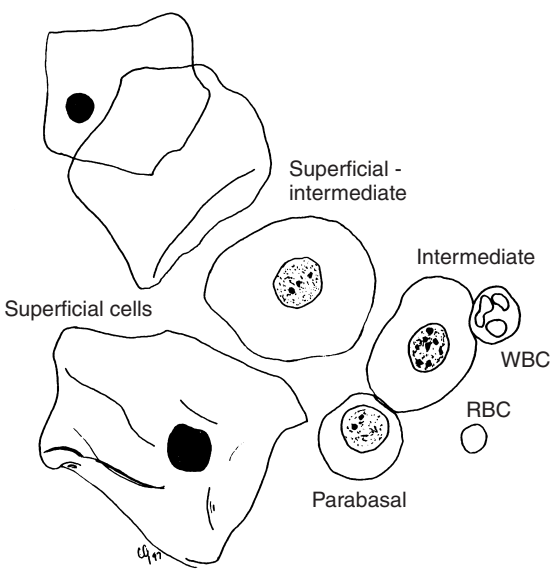

FIGURE 13-1. Typical cells visible in vaginal cytology preparation. *RBC,* Red blood cell; *WBC,* white blood cell.

TABLE 13-1. Vaginal Cytologic Findings During the Estrous Cycle

	PROESTRUS	ESTRUS	DIESTRUS
Parabasal	+	–	–
Intermediate	+	Rare	+
Superficial-intermediate	±	+	±
Superficial	Rare	90%	Rare
RBCs	+	±	±
WBCs	+	–	++/+

RBCs, Red blood cells; *WBCs,* white blood cells.

During proestrus, the parabasal, intermediate, and some superficial cells are exfoliated. RBCs, white blood cells (WBCs), and bacteria are present. As estrus approaches, the animal experiences a gradual increase in the maturity of the epithelial cells and a decrease in WBCs. During estrus, superficial cells predominate (eventually accounting for > 90% of the exfoliated epithelial cells). RBCs and extracellular bacteria are often present. WBCs are absent during estrus unless concurrent inflammation exists.

Diestrus (luteal phase) is marked by an abrupt change: parabasal and intermediate cells outnumber the superficial cells. Sheets of epithelial cells are often noted at the onset of diestrus. WBCs almost always return at this time. RBCs and bacteria are often present. Thus it is often impossible to base differentiation of proestrus from diestrus on a single vaginal smear. Fewer cells are exfoliated during anestrus. Parabasal and intermediate epithelial cells, with or without a few WBCs and bacteria, are present during anestrus (see Table 13-1). Similar changes occur during the feline estrous cycle, except that RBCs and WBCs are uncommon.

Breeding and Whelping Management

Determining the stages of the estrous cycle helps determine optimal breeding dates to maximize conception and litter size and to predict whelping dates more accurately. When using vaginal cytology as the only laboratory aid to breeding management, maximal conception rates and litter size are obtained when normal bitches are bred on the first day of estrus and again 3 or more days later. Although it may not change conception rates or litter size, breeding every other day is also acceptable, provided that breeding begins at the onset of estrus and continues for at least 4 to 6 days (Table 13-2). Because ovulation occurs at variable and unpredictable times during behavioral or cytologic estrus and because canine sperm maintains its ability to fertilize for at least 4 to 6 days in the estrual tract, normal gestation length varies from 58 to 72 days after the first breeding date. When gestation length is calculated from the first day of cytologic diestrus, 93% of bitches whelp 57 days after the first day of diestrus. Calculating gestation length from cytologic diestrus instead of breeding dates helps manage pregnancies.

Abnormal Cycles

If proestrus plus estrus lasts greater than 40 days in a bitch, it is considered prolonged. Because the time of ovulation during an abnormal cycle is unknown, breeding should continue every 2 to 4 days throughout the prolonged estrus, unless progesterone is being monitored to predict ovulation (see Progesterone, later). Persistent estrus is uncommon in bitches and queens; causes include ovarian cysts, ovarian neoplasia, and exogenous estrogen administration.

Some bitches have no apparent external or behavioral signs of proestrus or estrus (i.e., so-called "silent heat"). This might be identified prospectively by frequent (every 1 to 2 weeks) examination of vaginal cytology or retrospectively by determining serum concentrations of progesterone.

TABLE 13-2. Canine Breeding Management

1. Choose only healthy, *Brucella canis*-negative, normal animals for breeding.
2. Identify the first day of estrus.
   Begin early (day 3) in season (proestrus).
   Check the bitch every other day by behavioral (tease with experienced stud) and/or vaginal cytology examination.
   (NOTE: Serum progesterone can be used to more accurately predict ovulation and determine breeding dates.)
   (NOTE: Breeding a predetermined number of days after onset of proestrus is not optimal management.)
3. Breed on the first day of estrus.
4. Breed once again 3 or more days later.
   (NOTE: Breeding every other day for at least three times is also acceptable.)
5. Pregnancy examination is indicated 20 to 30 days later.
   If *pregnant,* discuss pregnancy and parturition management.
   If *not pregnant,* determine serum progesterone to assess ovulation and luteal function.

Progesterone concentrations of greater than 2 ng/ml (6 nmol/L) denote luteal function, which normally occurs during the 60 days after estrus in bitches.

Mismating

Vaginal cytology may determine when a bitch has recently been bred, because sperm heads (not intact sperm) are detected in vaginal smears from 68% and 50% of bitches bred 24 and 48 hours earlier, respectively. Absence of sperm does not rule out recent copulation. Vaginal cytology is indicated before administration of estrogens (i.e., mismating shot). If the cytology suggests diestrus, estrogens are contraindicated because pyometra occurs in 25% of bitches given estradiol cypionate (ECP) during diestrus.

NOTE: Administration of ECP to any bitch is not recommended. It may produce mild to fatal bone marrow suppression.

VULVAR DISCHARGES AND OTHER DISORDERS

Vulvar discharge is best evaluated by vaginal cytology (Olson et al, 1984b). One must first differentiate normal from abnormal discharges and then identify the cause. The morphologic characteristics and numbers of vaginal epithelial cells, RBCs, WBCs, and bacteria are noted, as is the presence of other elements (e.g., neoplastic cells, mucus, debris, uteroverdin, endometrial cells, macrophages). Many reproductive disorders cause abnormal vaginal cytologic findings, even when no vulvar discharge is present.

Hemorrhage

RBCs are common in normal and abnormal vulvar discharges (Table 13-3); their significance is determined by accompanying cells. Mature superficial epithelial cells with RBCs are expected during normal proestrus and estrus but may also occur because of exogenous estrogens or ovarian pathologic abnormalities (e.g., follicular cysts, estrogen-producing granulosa cell tumor). RBCs mixed with mucus are found in the normal postpartum vaginal discharge (i.e., lochia). When cytology reveals peripheral blood with intermediate and parabasal epithelial cells, a review of the history and physical findings is indicated. Causes of hemorrhage include subinvolution of placental sites, vaginal laceration, uterine and vaginal neoplasia, uterine torsion, and coagulopathies.

TABLE 13-3. Causes of Hemorrhagic Vulvar Discharge in the Bitch and Queen

With Mainly Superficial (Mature) Epithelial Cells
Normal proestrus, estrus, or early diestrus
Ovarian remnant
Ovarian pathologic condition (i.e., cystic follicles, functional ovarian tumor)
Exogenous estrogen
Without Superficial (Mature) Epithelial Cells
Normal lochia
Subinvoluted placental sites
Vaginal laceration
Neoplasia of vagina or uterus
Uterine torsion
Bleeding disorder

Subinvolution of placental sites is a postpartum disorder characterized by a bloody discharge persisting for longer than 8 to 12 weeks. It usually occurs in otherwise healthy primiparous bitches; the blood loss is rarely significant. Diagnosis is based on historical, physical, and cytologic findings. The diagnosis can be confirmed by a histopathologic assessment of placental sites, but such is rarely necessary. Vaginal lacerations are relatively uncommon but may occur from breeding trauma, dystocia, obstetric procedures, or vaginoscopy. If vaginal laceration is suspected, endoscopy is indicated.

Leiomyomas are the most common uterine and vaginal tumors in small animals, and hemorrhagic vulvar discharge (with or without a mass) is the most common sign. Leiomyomas do not readily exfoliate; therefore, neoplastic cells are rarely found cytologically. If neoplasia is suspected, careful digital vaginal palpation plus transabdominal uterine palpation for a mass are the procedures indicated next. After that, uterine radiography, ultrasonography, or vaginal endoscopy (or combination thereof) may be used to guide biopsy.

Uterine torsions usually occur in gravid females, near term. Abdominal pain is the prominent finding, and a hemorrhagic discharge is common. If torsion is suspected, the clinician can prove it by documenting malposition with uterine radiology and ultrasonography.

Coagulopathies rarely present with vulvar bleeding as the only sign; more common causes of vulvar bleeding should be ruled out first (see Table 13-3). If a bleeding disorder is

then suspected, a hemostatic profile (see Chapter 5) is indicated.

Bleeding can accompany any inflammatory process. If the WBC:RBC ratio is more suggestive of exudate than blood, an inflammatory disease should be sought. *Important:* Bloody vulvar discharge, even when associated with attraction of male dogs, does not always represent proestrus or estrus (see Table 13-3).

Purulent or Septic Discharge

WBCs, with or without bacteria, suggest inflammation. They are normally found in large numbers during the first 1 to 2 days of diestrus and in lesser numbers in normal lochia. Septic or purulent vulvar discharges may originate from the vulva, vestibule, vagina, or uterus. The source of a septic/purulent exudate determines the prognosis and must be determined. Vaginoscopy should determine if vulvitis or vaginitis is responsible; physical evidence of inflammation (e.g., hyperemia, edema, pain, mucosal lesions) should be obvious.

Finding vaginitis does not eliminate concurrent uterine pathology (Figure 13-2). If endoscopy suggests a uterine source of the discharge or if endometrial cells are found during cytologic evaluation, uterine involvement is probable and abdominal radiography and/or ultrasonography are indicated to look, for uterine disease such as pyometra. Knowing the stage of the estrous cycle is essential for evaluating uterine pathology. During the luteal phase (diestrus), a septic or purulent discharge can occur because of cystic endometrial hyperplasia-pyometra, pregnancy with concurrent uterine or vaginal infection, or impending abortion. If the discharge occurs postpartum, postabortion, or otherwise during anestrus, metritis or uterine stump granuloma and abscess should be considered. Predisposing causes of metritis, such as retained placenta, retained fetus, abortion, or obstetric procedures, should be sought (see Figure 13-2).

Neoplastic Cells

Vaginal and vulvar masses are indications for fine-needle aspiration. Vaginoscopy helps evaluate the extent of involvement and the external urethral orifice. Transmissible venereal tumors (TVTs) exfoliate readily. Bitches with TVTs are usually examined because of a mass protruding from the vulva (see Chapter 16). Vaginal cytology may also identify urethral transitional cell carcinomas and vaginal squamous cell carcinomas. Rarely, mammary gland adenocarcinoma and lymphoma involve external genitalia. If cytology does not allow definitive diagnosis of neoplasia, a histopathologic assessment of an incisional or excisional biopsy should. Additional diagnostic tests for staging are dictated by the tumor type.

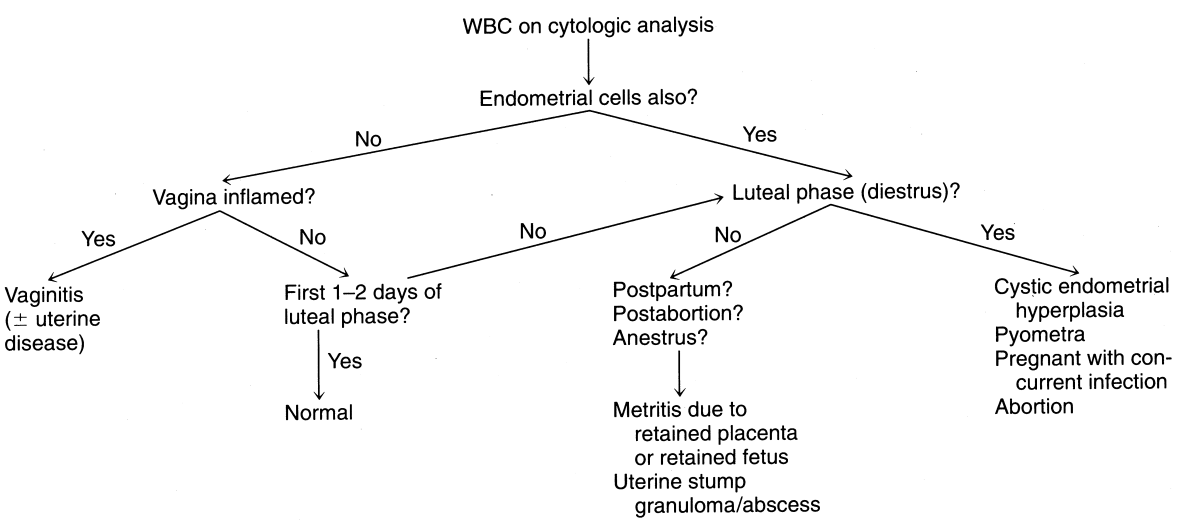

FIGURE 13-2. Diagnostic considerations for purulent or septic vaginal cytology. *WBC,* White blood cell.

Mucus

This is the predominate component of lochia. Mucoid discharge may also occur during normal late pregnancy and possibly in scant amounts during the nonpregnant, luteal phase (i.e., diestrus). No further testing of these animals is necessary. Cervicitis and mucometra can cause mucoid discharge; endoscopy or abdominal radiology/ultrasonography may diagnose these disorders, respectively. Some bitches with scanty mucoid vulvar discharge are apparently normal (Table 13-4).

Mibolerone (a synthetic androgen) or testosterone administration and intersex conditions may cause slight mucoid discharge. History should reveal drug administration. Clitoral enlargement commonly results from androgenic stimulation. The vagina ends blindly in some intersex animals. Measuring serum testosterone concentrations before and after human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone (GnRH) administration (see Testosterone, later) and finding a significant difference suggest functional testicular tissue in suspected intersex animals. These patients may also be karyotyped, but exploratory laparotomy may be the most cost-effective diagnostic and therapeutic approach.

Uteroverdine

This dark-green blood pigment is normal in the placenta. Its presence in vulvar discharge implies placental separation. Although normal during labor, its presence in the absence of obvious labor indicates fetal distress and dystocia or abortion. If history and physical examination do not differentiate these causes, abdominal ultrasonography is indicated. The presence of fetuses and their viability can be determined ultrasonographically.

TABLE 13-4. Causes of Mucoid Vulvar Discharge

Normal
Lochia
Late pregnancy
Luteal phase (diestrus)
Androgenic stimulation
Endogenous (intersex)
Exogenous (mibolerone, testosterone)
Cervicitis
Mucometra
Idiopathic (?)

MAMMARY GLANDS

Abnormal gland size or abnormal secretions from one or more glands usually reflect disorders of the mammae. The most common cause of mammary gland enlargement and secretion is lactation (which may be normal or abnormal; see later), but mastitis, hyperplasia, and neoplasia are also common.

Galactorrhea

Inappropriate lactation unassociated with pregnancy and parturition is common in bitches with false pregnancy and occasionally occurs in false-pregnant queens. False pregnancy occurs at the end of the luteal phase (i.e., approximately 60 days after estrus in a bitch; approximately 40 days after induced ovulation without conception in a queen). Galactorrhea may also occur after withdrawal of progesterone stimulation, such as oophorectomy during diestrus or discontinuation of an exogenous progestin (e.g., megestrol). Galactorrhea is diagnosed by historical and physical findings. It resolves spontaneously but may rarely persist for months in some bitches. Testing is usually unnecessary, but galactorrhea has been associated with hypothyroidism, making thyroid function testing (see Chapter 8) reasonable in bitches with protracted cases.

Mastitis

A bacterial infection of one or more lactating mammary glands, mastitis is most common in postpartum bitches. It rarely occurs in false-pregnant lactating bitches or postpartum queens. The diagnosis is based on a history of recent parturition plus finding warm, swollen, painful glands with purulent milk. If the milk is not visibly abnormal, cytology of the milk or a mammary aspirate shows purulent inflammation, which is often septic. Galactostasis (i.e., accumulation and stasis of milk) also causes warm, swollen, painful glands, but no bacterial infection is seen and the milk is cytologically normal. Galactostasis is most common during weaning.

Mammary Hypertrophy

Mammary hypertrophy (i.e., mammary hyperplasia, fibroadenomatous change, fibroadenoma) occurs more often in cats than dogs. Characterized by rapid abnormal mammary

growth temporally associated with progestational stimulation, mammary hypertrophy occurs during the luteal phase of the estrous cycle of intact bitches and queens. It has also been reported in neutered queens and toms treated with progestins (e.g., megestrol acetate [Ovaban]). If the history confirms progesterone stimulation, mammary hypertrophy is suspected. If one is in doubt, biopsy is indicated to distinguish it from neoplasia.

Mammary Neoplasia

Mammary neoplasia is common in middle-aged to older female dogs and cats. Any mammary growth in male or neutered animals or intact anestrus females is considered neoplastic until proven otherwise. Fine-needle aspiration of the mass is easy; however, cytologic findings must be interpreted cautiously (see Chapter 16) and are often nondiagnostic. Excisional biopsy and a histopathologic assessment best indicate the type and degree of malignancy.

SEMEN EVALUATION

Common Indications • Semen evaluation is indicated whenever there is a possibility of male infertility (Figure 13-3); it is also routine in prebreeding examinations. Cytology of the ejaculate is also used to evaluate prostatic, testicular, and epididymal diseases.

Technique • Semen is collected by manual stimulation of the dog's penis through the prepuce or an artificial vagina. Most dogs with previous breeding experience can be collected without a teaser bitch. Inexperienced or timid males may need a bitch in estrus to ejaculate. A docile anestrus bitch may also work as a teaser. The presence of an estrual bitch or use of the synthetic pheromone methyl-p-hydroxy benzoate (Sigma Chemical) may improve the ejaculate quality. The collection area should be quiet, free from distractions, and have a nonskid floor.

Care should be taken when handling the semen sample. All equipment should be clean and free of contaminants (e.g., water, excessive lubricant) that may affect sperm viability. The sample should be protected from temperature shock. Dog semen can be handled at room temperature for approximately 15 minutes without adverse effect. Nevertheless, the sample should be processed promptly. Slides and coverslips should be maintained at 37° C. The semen sample is evaluated for volume and color, as well as concentration, motility, and morphology of spermatozoa (Table 13-5).

Volume • The volume is read directly from the calibrated centrifuge tube into which the sample was collected. Canine semen is ejaculated in three fractions. The first fraction is clear; volume is usually several drops. Some individuals may have up to 2 ml of this fraction. The second fraction is sperm-rich; volume is from 0.5 to 5 ml. The third and largest fraction (occasionally 20 ml) is prostatic fluid. Normal prostatic fluid is clear and easily differentiated from the milky sperm-rich fraction. For routine semen evaluation, the clinician should collect only enough prostatic fluid to ensure that the entire sperm-rich portion has been ejaculated.

The total volume varies with technique, frequency, age, and the amount of prostatic fluid collected. Young dogs usually have less volume than mature dogs. Volume is not correlated with fertility unless the dog fails to ejaculate.

Color • The color is evaluated by direct visualization. Normal canine semen is usually the color of skim milk. An abnormally colored sample should be closely examined for foreign matter; contaminants may decrease sperm viability and concentration. A yellow appearance may represent urine contamination. To avoid this problem, dogs should not be allowed to micturate immediately before semen collection. Rarely, dogs urinate during ejaculation; they are usually infertile. Blood colors the sample pink or red and is usually of prostatic origin or from penile abrasions. The latter is identified by prompt visual inspection of the penis.

Inflammatory cells may cause the sample to appear flocculated or yellow-green. These cells can originate anywhere in the urinary or reproductive tracts. Preputial contamination is common. When leukospermia occurs, the source should be sought and the sample cultured for bacteria and *Mycoplasma spp*. Dogs should be tested for *B. canis* infection (see Chapter 15).

Concentration • The volume and thus the concentration of the ejaculate is principally influenced by the sperm-free prostatic fluid. Therefore the number of spermatozoa is

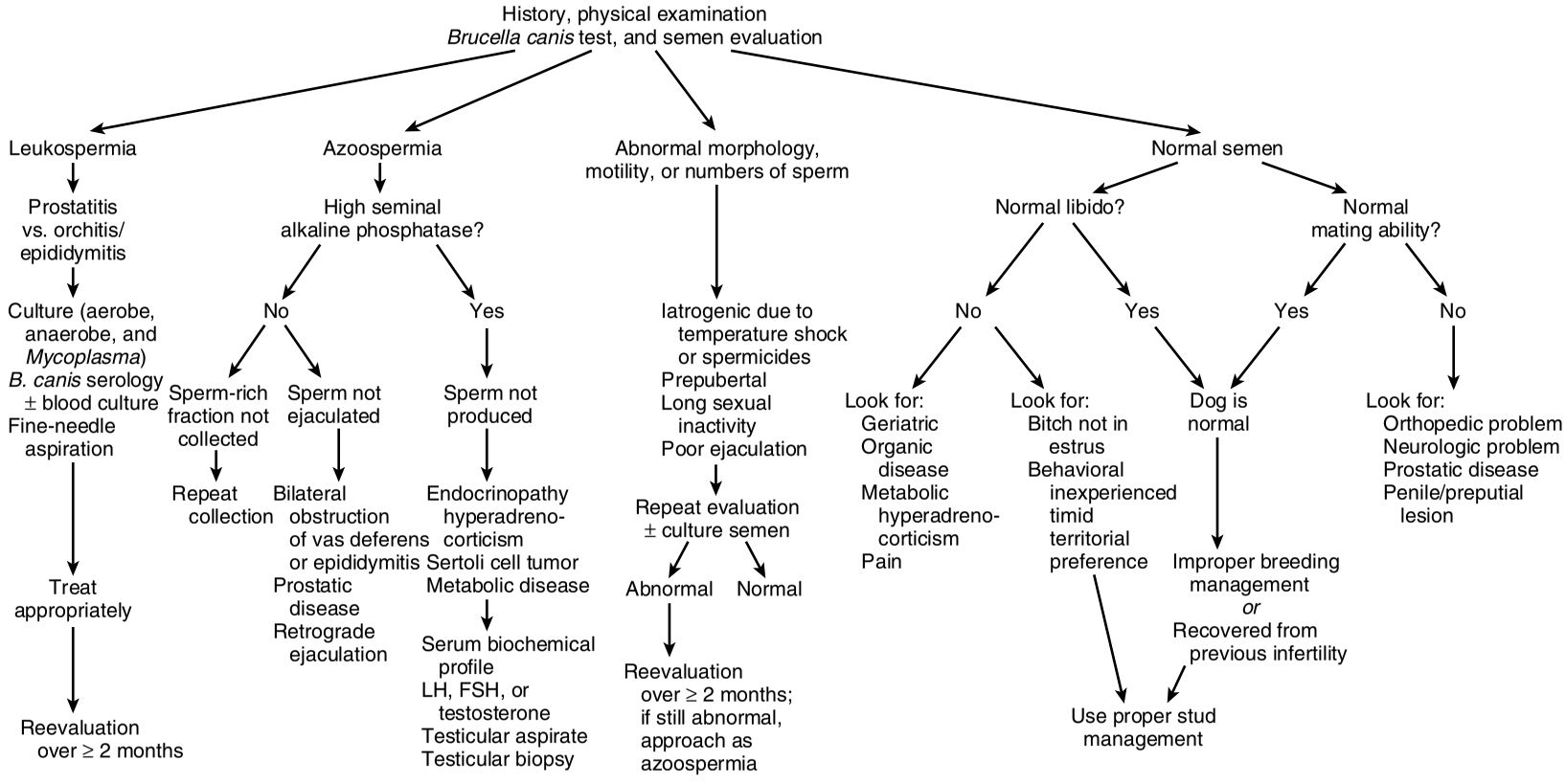

FIGURE 13-3. Diagnostic approach to male infertility. *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone.

TABLE 13-5. Characteristics of Normal Canine Semen

FRACTION	VOLUME	COLOR
Presperm	Drops (rarely several milliliters)	Clear
Sperm-rich	0.5-5.0 ml	Cloudy white, opalescent
Prostatic fluid	1-20 ml	Clear

Total sperm/ejaculate, 250×10^6 to 2000×10^6.
Motility ≥ 80% progressively motile.
Morphology ≤ 25% abnormal.

reported as the total sperm per ejaculate, not sperm per milliliter. An easy way to count spermatozoa is using a hemocytometer (see Chapter 2). Either the RBC or the WBC and platelet Unopette may be used for canine semen. The RBC pipette dilutes the sample 1:200. The WBC and platelet pipette dilutes the sample 1:100. Dilution may be unnecessary for oligospermic samples. The number of sperm per milliliter is then multiplied by the number of milliliters collected to give the number of sperm per ejaculate. Values of 250 to 2000×10^6 sperm per ejaculate are normal. Breed variation is considerable because sperm production is directly related to testicle size.

Motility • A drop of semen is placed on a warm slide (37° C), covered with a warm coverslip, and allowed to sit on the slide warmer for 15 to 30 seconds. It is then examined microscopically for progressive motility. Spermatozoa should move in a rapid, steady, forward manner, straight across the field. Dead sperm may be bounced back and forth by live sperm, especially in a concentrated sample. This passive movement must be distinguished from active motility. A concentrated sample should be diluted with an equal volume of 0.9% saline (37° C). One hundred sperm are examined, and the percentage having progressive motility is estimated. Seventy to 75% motility is expected, but dogs with good fertility usually have greater than 80%. Wave patterns (e.g., as seen in bull semen) are rare in canine semen, because the sperm concentration is too low.

Exposure to heat or cold, excessive lubricant, water, urine, and inflammatory cells can diminish the percentage and vigor of motility. Motility of sperm ejaculated after a long sexual rest may be lower. Chilled semen that is rewarmed usually has the same percent of motile sperm; however, the individual spermatozoa tend to move slowly. In semen that has been frozen and then thawed for artificial insemination, both the percentage and speed of motility are usually lower.

Morphology • Phase contrast light microscopy is ideal for evaluating sperm morphology but has limited availability. Several stains may be used to evaluate the morphologic characteristics of sperm. Unfortunately, some stains cause morphologic defects (especially bent tails) in canine sperm. These defects may reflect inappropriate osmolality or pH. The most common stains contain eosin-nigrosin. Samples may also be stained with NMB or Wright's stain. Sperm take up stain more slowly than do blood cells, so staining times usually need to be lengthened by several seconds to minutes. An inexpensive stain is Pelican India ink, available from art stores. A three-slide technique is used. One drop of the ink is mixed with one drop of semen on a slide. A second slide is drawn back to touch the edge of the drop. This fluid is then drawn across a third slide with the edge of the second slide to make a thick tapering to a thin smear (similar to how a blood smear is made). The slides are air dried and examined microscopically under oil immersion.

At least 100 (preferably 200) spermatozoa are examined and classified as being normal or abnormal. If a spermatozoon has more than one abnormality, it is classified according to the most severe abnormality. Abnormalities affecting the size and shape of the sperm head, acrosome, and midpiece and proximal droplets are usually considered most severe. Loose or detached heads (that are otherwise normal) and bent tails are considered less severe; however, they are often the first abnormalities noticed after testicular insult. The correlation between morphologic characteristics and fertility has not been thoroughly evaluated.

Primary abnormalities are usually attributed to abnormal testicular spermatogenesis, whereas secondary abnormalities are usually attributed to epididymal maturation process errors or sample handling. This assumption can be inaccurate; therefore, it is best to describe the abnormalities (i.e., bent tail, large head) rather than merely call them primary or secondary. In this way, comparison with future samples may be more accurate. Dogs with good fertility usually have less than 20% to 25% abnormal sperm. Abnormalities resulting from improper sample handling should

disappear in subsequent, properly handled samples. The most common iatrogenic change in canine semen is bent tails.

During the morphologic examination, the presence of other cell types or foreign matter should be recorded. RBCs denote hemorrhage, and WBCs imply urogenital tract inflammation. Some epithelial cells are normally present in canine semen, and their numbers may increase after a long sexual rest. If excessive numbers of cells other than spermatozoa are found, their source should be investigated. These cells are most easily evaluated if stains such as Wright's are used. Crystals are a common type of foreign matter. They may be found in samples contaminated with urine or talc.

Seminal Alkaline Phosphatase

Alkaline phosphatase is produced by the canine epididymis and is found in high concentrations (i.e., >1000 IU/L) in normal ejaculates. Determination of seminal plasma alkaline phosphatase helps evaluate azoospermic semen samples. The sample is centrifuged as for serum (e.g., 3000 rpm for 10 minutes), and the supernatant seminal plasma is harvested. Centrifugation can be modified depending on the sample's viscosity, but canine semen is rarely more viscous than serum. The concentration of alkaline phosphatase in the seminal plasma is then determined in the same manner as for serum alkaline phosphatase (see Chapter 9). If the sperm-rich fraction was not contained in the semen sample (i.e., incomplete ejaculation or bilateral obstruction to outflow), seminal alkaline phosphatase will be low (i.e., <300 IU/L). If the sample has high alkaline phosphatase but no spermatozoa, testicular spermatogenesis failure is the problem. Azoospermic animals with seminal alkaline phosphatase in the midrange require additional testing.

Interpretation • The semen sample does not reflect testicular or epididymal function on the day the sample is collected. Spermatogenesis requires approximately 60 days in dogs. The length of the spermatogenic cycle in cats is unknown. To help establish a prognosis or resolve doubt about the cause of an unsatisfactory sample, the dog should be reevaluated several times over at least 2 months. A dog's age, breed (testicle size), and frequency of use must be considered before pronouncing a sample "satisfactory" or "unsatisfactory." Finding semen of satisfactory quality is not proof of fertility; a male must also have normal libido and mating ability (see Figure 13-3). Likewise, the presence of unsatisfactory semen does not necessarily signify sterility, unless azoospermia or complete necrospermia (i.e., dead spermatozoa) is found.

PROSTATE

Prostatic disease (e.g., benign prostatic hyperplasia, bacterial prostatitis, prostatic abscess, prostatic and paraprostatic cysts, prostatic neoplasia) is common in older male dogs. The history and physical examination (especially rectal palpation) often localize disease to the prostate, but additional tests are necessary to determine the cause. Prostatic fluid is the third and largest fraction of the canine ejaculate; therefore, evaluation of ejaculated prostatic fluid has been advocated for the diagnosis of prostatic disease. Prostatic massage, fine-needle aspiration, and biopsy of the prostate can also be performed, but ejaculation is less invasive and easily performed if a dog is willing and able to ejaculate.

Ejaculate characteristics correlate well with histopathologic examination of biopsy specimens, except that neoplastic cells are rarely found in the ejaculate of dogs with prostatic neoplasia. Hemorrhage is the most frequent abnormality in semen from dogs with prostatic hyperplasia, whereas inflammation is the most frequent abnormality from dogs with chronic bacterial prostatitis. Because prostatic fluid normally refluxes into the urinary bladder, urinary tract infection frequently develops secondarily to chronic bacterial prostatitis. When urinary tract infection coexists with bacterial prostatitis, semen cytology is a more accurate diagnostic test for prostatitis than is prostatic massage.

Percutaneous fine-needle prostatic aspiration and biopsy, especially with ultrasonographic guidance, are excellent methods of obtaining material for microbiologic and cytologic evaluation. Radiology and ultrasonography are also useful. Unlike with people, biochemical evaluation of canine prostatic fluid has not helped evaluate various prostatic diseases. Serum and seminal prostate-specific antigen and prostatic acid phosphatase are unchanged in dogs with prostatic disease. Canine prostate-specific esterase (CPSE) is increased in dogs with benign prostatic hyperplasia relative to

normal dogs, but it is unknown whether changes in CPSE accurately differentiate prostatic diseases.

TESTES AND EPIDIDYMIDES

The most important diagnostic procedure for evaluation of the testes and epididymides is physical examination, but semen evaluation is also useful. For example, abnormal sperm (i.e., deformed acrosomes, swollen midpieces, retained protoplasmic droplets) are ejaculated as early as 5 weeks after infection with *B. canis* (George et al, 1979). By 15 weeks after infection, bent tails, detached heads, and head-to-head agglutination are also found. Between 8 and 35 weeks after *B. canis* infection, clumps of inflammatory cells occur in semen (i.e., neutrophils and macrophages) plus phagocytosis of sperm. With chronic infection (i.e., 60 to 100 weeks), fewer inflammatory cells are found. When testicular atrophy results from *B. canis* infection, azoospermia is likely. Any dog with infertility or inflammatory cells in the semen (leukospermia) should be evaluated for *B. canis* with serology and microbiology (see Chapter 15).

Fine-needle testicular aspiration is easily performed using a 25-gauge needle and is especially helpful in evaluating focal lesions (i.e., palpable testicular masses). It may also be performed to demonstrate the presence of spermatogenesis. Aspiration of the epididymides causes more discomfort and may result in spermatic granuloma formation.

ENDOCRINE EVALUATION

History and physical examination provide the most important diagnostic information when evaluating the hypothalamic-pituitary-gonadal axis. Normal libido and mating behavior in both sexes, normal cyclicity in females, and a physically normal reproductive tract suggest a normal hypothalamic-pituitary-gonadal axis. The clinician can substantiate this by demonstrating normal semen in males and normal ovulations in females. Noninvasive procedures to prove that ovulation occurred are not available. High serum progesterone concentration suggests luteal function, however, which strongly suggests that ovulation occurred.

Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, and testosterone are measured using radioimmunoassay (RIA). Normal ranges vary considerably from laboratory to laboratory. Reproductive hormones are released in pulsatile or cyclic manners; therefore, results must be interpreted in conjunction with each other and the reproductive history. Serial determinations (e.g., every 15 to 30 minutes for 2 hours) or measurement before and after administration of trophic hormones is preferable to single, random blood samples (Shille and Olson, 1989).

Progesterone

Common Indications • Measurement of serum progesterone concentration is used to predict and confirm ovulation, assess remnant ovarian tissue, monitor luteal function, and predict parturition. Progesterone concentrations help predict ovulation in bitches because luteinization begins as a preovulatory event in dogs. The transition of progesterone concentration from anestrus levels of less than 1 ng/ml to greater than 2 ng/ml (6 nmol/L) occurs during the LH surge. Ovulation follows the LH surge by about 2 days. The oocyte completes its development and is ready for fertilization approximately 2 days after ovulation. Therefore insemination is recommended 3 to 4 days after the transition from anestrus progesterone to concentrations greater than 2 ng/ml. Pregnancy rates have been best when insemination is performed when progesterone concentrations are greater than 8 ng/ml up to 19 to 26 ng/ml (60 to 80 nmol/L). Progesterone concentrations greater than 8 ng/ml (25 nmol/L) imply that ovulation has occurred. Measuring progesterone every 2 to 3 days is usually adequate.

Serum progesterone concentrations greater than 2 ng/ml (6 nmol/L) are necessary to maintain pregnancy in bitches. Parturition begins within 24 hours of progesterone's dropping below 2 ng/ml. Because progesterone is thermogenic, a drop in rectal temperature to less than 100° F reflects a drop in concentration. Therefore, progesterone concentrations can help predict parturition and manage dystocia. Parturition is expected 64 ± 1 days after the preovulatory rise (i.e., LH surge) and within 24 hours of the prepartum decline to less than 2 ng/ml. Monitoring serum progesterone helps determine the efficacy of abortifacients and treatment of luteal cysts with prostaglandins. In both situations,

progesterone must be less than 2 ng/ml to ensure treatment success.

Sample • The clinician should check with the laboratory (serum or plasma). Heparinized and ethylenediaminetetraacetic acid (EDTA) plasma yield similar results to serum. RIA and enzyme-linked immunosorbent assay (ELISA) methods are used.

Artifactual Changes • Storage on RBCs or at room temperature can decrease concentrations. Serum progesterone is unaffected by lipemia, icterus (i.e., bilirubin < 20 mg/dl), or hemolysis when RIA is used. RIAs are highly specific for progesterone but may slightly cross-react with deoxycorticosterone, deoxycortisol, dihydroprogesterone, or 5β-pregnan-3, 20-dione. This assay should not cross-react with androstenediol, corticosterone, cortisol, estradiol, testosterone, or pregnenolone. Cross-reactivity may vary with specific assay systems. Administration of radioactive compounds or exogenous progestins may affect the RIA directly. Exogenous progestins are likely to suppress endogenous progesterone via negative feedback.

ELISA progesterone determinations are approximately 85% accurate when compared with RIA. Hemolysis and lipemia interfere with ELISA.

Interpretation • The importance of using all the data rather than one parameter alone is well illustrated with progesterone. Progesterone concentrations greater than 2 to 8 ng/ml suggest luteal function, which implies but does not prove that ovulation occurred. In a bitch, luteal function normally lasts for approximately 65 days after estrus, whether or not pregnancy occurs. If pregnancy does not occur, luteal function persists for approximately 40 days in queens; if pregnancy does occur, progesterone remains greater than 2 ng/ml to term. Serum concentrations of progesterone are highest approximately 20 to 30 days after ovulation, at which time it may be greater than 50 ng/ml (i.e., > 75 nmol/L), depending on the laboratory. At all times of the normal estrous cycle other than the luteal phase, progesterone concentrations are less than 2 ng/ml, because (normally) no functional luteal tissue exists. Therefore serum concentrations of less than 2 ng/ml in a patient may reflect normal anestrus, failure to ovulate, failure to maintain normal luteal function, or eminent parturition, depending on when in the cycle the sample was obtained (Figure 13-4). Conversely, serum concentrations greater than 2 ng/ml may indicate that (1) ovulation has occurred, (2) luteal function is normal, (3) ovarian tissue is still present in a previously spayed animal, (4) luteal cysts or progesterone-producing granulosa cell tumors are present, (5) prostaglandin therapy (for pyometra, induced abortion, or luteal cysts) has not yet been effective, or (6) parturition is not imminent.

Testosterone

Uncommon Indications • Serum testosterone can be measured to evaluate suspected cryptorchid or intersex animals and to identify the cause of infertility in certain cases.

Sample • The clinician should check with the laboratory. EDTA plasma may yield lower results than heparinized plasma or serum with RIA.

Artifactual Change • Storage on RBCs or at room temperature can decrease concentrations. Testosterone is unaffected by hyperbilirubinemia (i.e., < 20 mg/dl), hemolysis, and lipemia. RIA is highly specific for testosterone; however, there may be important cross-reactivity with dihydrotestosterone, 19-nortestosterone, 11-ketotestosterone, methyltestosterone, and 11-β-hydroxytestosterone. Cross-reactivity with aldosterone, corticosterone, cortisol, cortisone, estradiol, and progesterone should be insignificant. Cross-reactivity may vary with the reagents and assay system used.

Administration of radioisotopes, exogenous testosterone, or drugs metabolized to testosterone, methyltestosterone, or other cross-reacting androgens listed earlier may affect the RIA directly. Exogenous androgen treatment may suppress endogenous testosterone.

Interpretation • Serum testosterone concentrations fluctuate greatly within and among individuals. Pulses of testosterone secretion occur about every 30 to 90 minutes, depending on the species. Determination of testosterone on a single, random occasion has no diagnostic value (i.e., normal, fertile male cats may have undetectable amounts during the day). Testosterone should be

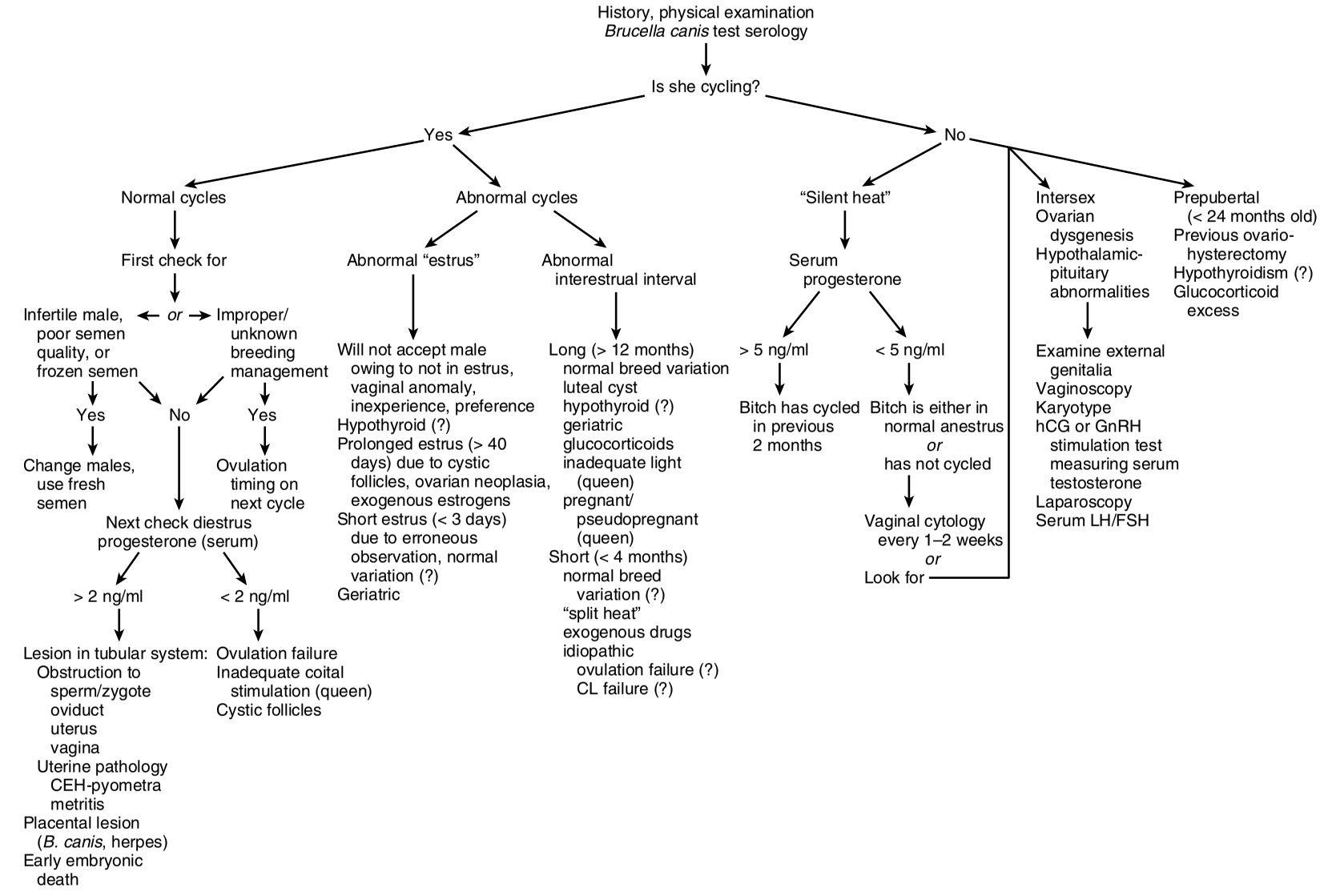

FIGURE 13-4. Diagnostic approach to infertility in a bitch. *CEH,* Cystic endometrial hyperplasia; *CL,* corpus luteum; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone.

determined before and after administration of hCG or GnRH; substantial increase in testosterone signifies functional testicular tissue. A widely accepted protocol for hCG or GnRH stimulation testing in dogs and cats has not been established. Therefore, consultation with the laboratory performing the assay is recommended to determine the dose of hCG or GnRH to administer to the patient, frequency of sampling, and interpretation of results (see Appendix I).

Luteinizing Hormone

Uncommon Indications • Measurement of serum luteinizing hormone is done to predict ovulation and evaluate pituitary responsiveness. Testosterone or progesterone concentrations are usually evaluated simultaneously with LH to assess the pituitary-gonadal axis after GnRH administration.

Sample • LH is labile; serum must be promptly removed from RBCs and kept frozen until assayed. LH can be measured before and after GnRH administration. The clinician should contact the laboratory for recommended dose and sampling protocol. RIA and ELISA methods are used.

Artifactual Change • Cross-reactivity exists between the LH assay and equine chorionic gonadotropin (eCG) but not with hCG. Hemolysis, icterus, and lipemia do not interfere with the RIA assay, but they render the ELISA assay useless.

Interpretation • A single determination of basal LH is not helpful except to assess an absence of gonadal hormone negative feedback. LH concentrations increase above the ranges of sexually intact animals within days of spaying or castrating. Some dogs with testicular failure also have increased LH concentrations. LH should increase after GnRH administration (the magnitude depending on the stage of the estrus cycle). After the increase in LH, testosterone or progesterone concentrations should increase if the pituitary-gonadal axis is normal. Serial determination of LH beginning during proestrus can identify the preovulatory LH surge in bitches. Because the duration of the surge is usually only 12 to 24 hours, measurements must be made at less than or equal to 24-hour intervals. Ovulation is expected approximately 2 days after the LH surge, and canine oocyte maturation is completed about 2 days later. Therefore, insemination is timed for about 4 days after the LH surge to optimize conception and litter size in dogs. The accuracy of the ELISA LH assay relative to the RIA has not yet been reported. The clinical usefulness of the ELISA LH assay relative to ELISA or RIA progesterone assays for ovulation timing in the bitch remains to be determined. The need for frequent sampling makes it inconvenient and expensive.

Estradiol

Rare Indications • Evaluation of serum concentrations of estradiol is rarely indicated in dogs and cats because the readily available RIA assays do not usually cover the appropriate range of serum concentrations found in those species (see later) and because the common conditions associated with pathologic production of estradiol are usually easily evaluated by other methods. The most common conditions associated with abnormal estradiol production are testicular tumors in male dogs and ovarian remnant syndrome and follicular ovarian cysts in female dogs and cats.

Testicular tumors are best identified by using palpation, ultrasound examination, or both to examine the testes. Ovarian remnant syndrome is characterized by failure to stop cycling, continuing to exhibit estrous behavior, or resumption of estrous cycles and estrous behavior after ovariohysterectomy. Finding vaginal cornification coincident with classic behavioral or physical signs of estrus is diagnostic. Cystic follicles can cause persistent estrus or abnormally frequent estrous cycles. Ovarian ultrasonography should be diagnostic.

Sample • Serum is used. Storage on RBCs and at room temperature may decrease the concentration. RIA is used.

Artifactual Changes • Insignificant cross-reactivity exists between estradiol-17β and other endogenous steroid hormones, except estriol and estradiol-17α. Administration of radioisotopes and exogenous estrogens may directly affect the assay. Cross-reactivity varies with the assay system.

Interpretation • Normal serum concentrations in dogs and cats are at or below the level of detection for the assays. Therefore, normal

and abnormal conditions cannot always be differentiated. Vaginal cytology detects changes in the vaginal epithelium associated with estrogen and is a convenient bioassay for estradiol in dogs and cats.

Relaxin

Uncommon Indications • Measurement of serum relaxin is done in the bitch to determine pregnancy, to differentiate false pregnancy from pregnancy, and perhaps to assess spontaneous abortion. Relaxin is produced primarily by the placenta and is first detectable in the blood during the fourth week of pregnancy. It is highest during weeks 6 to 8 and then declines (although it is present in serum and milk during lactation).

Sample • RIA, ELISA and rapid immunomigration (RIM) methods are used. The commercially available, patient-side RIM (Witness® Relaxin; Synbiotics) test can be performed on serum or plasma but not whole blood. Samples must be assayed within 4 hours if held at room temperature or within 48 hours if refrigerated at 2° to 7°C (35° to 45°F). Hemolysis may obscure a weak positive line, although it does not significantly interfere with the test per se.

Interpretation • Relaxin is detectable about 22 to 27 days after breeding (26 to 31 days after the LH surge). This is about the same time that either abdominal palpation or ultrasonography can confirm pregnancy. Because the test is very specific and because relaxin is produced primarily by the placenta, a positive test result indicates pregnancy. False positive results have not been reported. A sudden disappearance of relaxin from blood (i.e., suddenly a negative result) would indicate that spontaneous abortion occurred. False-negative results occur when the test is performed too early during gestation. It is also possible that a small litter size could yield a false-negative result. Relaxin is similarly produced by pregnant queens but in lower concentrations. At the time of this writing, the commercially available relaxin test had not been validated for accuracy in cats.

Miscellaneous

Assays for FSH are not readily available at this time.

ANCILLARY TESTS

Animals suspected of having intersex conditions or abnormal gonadal development may be karyotyped. Fine-needle aspirates are easily obtained from the testes, epididymides, prostate, and from vaginal lesions. Biopsy specimens can be obtained from any part of the reproductive tract, although gonadal biopsy might have deleterious effects on remaining normal tissue. Testicular biopsy has not changed semen quality in normal dogs. Certain acute-phase reactant proteins, including fibrinogen, are increased during pregnancy. Therefore, it has been suggested that fibrinogen be used for pregnancy diagnosis in dogs. The finding of *increased fibrinogen is not specific for pregnancy*, because it can be associated with a wide variety of inflammatory conditions.

Complete Blood Count

The packed cell volume (PCV) of pregnant bitches and queens declines after about day 20 of gestation and continues to decline until parturition, when PCVs of 30.6% ± 0.8 can be expected in bitches. Pregnant animals may have mild, mature neutrophilia. Dogs are very sensitive to the toxic effects of estrogens on bone marrow stem cells. Thrombocytopenia, leukopenia, leukemoid reactions, anemia, or a combination thereof can occur because of pharmacologic doses of estrogens and estrogen-producing Sertoli or granulosa cell tumors (see Chapter 3).

Neutrophilic leukocytosis with a left shift, variable degrees of neutrophil toxicity, and monocytosis are commonly associated with mastitis, metritis, and prostatic abscesses. The leukogram of animals with pyometra varies, although leukocytosis with a left shift is expected. Profound leukocytosis (i.e., 100,000 to 200,000/µl) with a left shift may occur because of a closed cervix pyometra. Conversely, leukopenia with a degenerative left shift may occur in animals with severe sepsis as the result of pyometra or prostatic abscessation causing generalized peritonitis.

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14

Joane Parent

Neurologic Disorders

DIAGNOSTIC APPROACH

The most important step in diagnosing central nervous system (CNS) disease is the neurologic examination. The objective is to localize the lesion, trying to account for all abnormalities with one lesion. If this is not possible, a multifocal or diffuse disease is diagnosed. Multifocal or diffuse CNS diseases clinically often present with deficits that relate to one lesion. A routine approach should be followed when performing the examination and the findings recorded. This avoids missing any steps of the neurologic examination and allows for a more objective subsequent reevaluation.

Guidelines for Lesion Localization

Abnormal mental status suggests intracranial disease (i.e., thalamocortical disorders, brain stem disorders). Behavioral change or seizure activity suggests thalamocortical disease, whereas altered consciousness (e.g., somnolence, stupor, coma) suggests brain stem (i.e., midbrain, pons, medulla) abnormalities. The history is crucial in the evaluation of the mental status. Because of excitement, apprehension, or fear, the behavior displayed by the animal in the examination room rarely represents the mental status observed in the home environment.

Evaluation of the gait and posture may detect ataxia. Ataxia (i.e., deviation of the animal's body from the main axis, the vertebral column) can be caused by problems in the cerebellum, vestibular system, or ascending proprioceptive pathways. Vestibular ataxia causes concomitant head tilt. Cerebellar ataxia occurs in all limbs if the lesion is bilateral or on the ipsilateral side if it is unilateral. In cerebellar diseases, no proprioceptive deficits exist because ascending proprioceptive pathways to the cortex are intact, and strength is preserved because the descending motor pathways from the cortex to the lower motor neurons are also intact. These animals are characteristically "bouncy" when ambulating. Proprioceptive ataxia caused by disease of ascending proprioceptive pathways in the spinal cord is accompanied by weakness because of concomitant involvement of the descending motor pathways. Proprioceptive ataxia caused by disease of the brain stem is accompanied by somnolence and cranial nerve deficit or deficits.

Cranial and spinal nerve reflex deficits help localize lesions along the neuraxis, the cranial nerve reflexes for the brain stem, and the spinal reflexes for the spinal cord. Counting mentally from 1 to 12 is the best way to include all cranial nerves while performing an examination. Funduscopic examination is vital because the optic nerve is a direct projection of brain tissue; the eyes may be involved by extension of a disease process (e.g., feline infectious peritonitis, canine distemper). Postural reactions are nonlocalizing

by themselves. With thalamocortical disease, these reactions may be absent contralateral to the lesion despite a normal gait. Pain perception is important for prognosis. The anatomic diagnosis is derived from the abnormalities observed on the neurologic examination.

The anatomic diagnosis, history, and signalment are combined to establish the differential diagnosis. History can be invaluable (e.g., road accident, known exposure to toxin). The signalment is important because many neurologic disorders have a breed predisposition or a genetic cause (e.g., intervertebral disk disease of Dachshunds, cervical vertebral instability of Dobermans). Clinicians should check recent neurology texts when treating unusual breeds with neurologic disease.

The physical examination, complete blood count (CBC), serum biochemical profile, and urinalysis reveal if the disorder might involve other body systems. It also helps the clinician to evaluate the general health of a patient before general anesthesia, which is often required for other tests. When data base abnormalities are present, it is important to seek the origin before more invasive testing. As an example, the possibility of a tick-borne disease should be investigated if thrombocytopenia is observed in an animal with intracranial disease. When the nervous system is affected secondarily to a metabolic or toxic disorder, the abnormalities are usually bilateral and symmetric (e.g., polyneuropathies, generalized seizure).

The cerebrospinal fluid (CSF) analysis was long considered the most valuable test for evaluating intracranial disease, especially if the history was suggestive of an inflammatory disorder. With the increasing availability of magnetic resonance imaging (MRI), this is changing. Marked CSF pleocytosis may be observed with space-occupying lesions such as in meningioma or intracranial arachnoid cyst. Without neuroimaging, a diagnosis of primary inflammatory brain disease would be posed, yet MRI would unequivocally indicate the presence of a space-occupying lesion. On the other hand, a normal CSF cell count may be seen with diffuse periventricular edema on MRI, suggesting viral encephalitis. Whenever possible, the combination of CSF analysis and MRI is the ideal diagnostic approach regardless of the intracranial disease suspected. Computed tomography (CT) and MRI are preferred over CSF analysis when intracranial space-occupying lesions are suspected. For spinal cord disorders, survey radiographs, CSF analysis, myelography, or a combination thereof are indicated. It is likely that MRI will gradually replace the more invasive myelography. When the disorder affects the peripheral nervous system, electromyography and fascicular nerve and muscle biopsies are the most useful tests. Electrodiagnostics are more rewarding for peripheral nervous system lesions than for CNS disorders. For CNS lesions, these tests are more useful for prognosis and evaluation of response to treatment.

CEREBROSPINAL FLUID ANALYSIS

Frequent Indications • CSF analysis requires general anesthesia. CSF is the only readily accessible tissue that evaluates current CNS status and is warranted every time a CNS disorder is suspected. Primary CNS inflammatory diseases rarely cause CBC or serum biochemical profile changes. If CBC or profile changes occur, a multisystemic disorder with secondary CNS involvement is likely. CSF analysis is indicated even if the CBC and profile are normal. Repeated CSF analysis can help evaluate response to treatment and obtain baseline data before discontinuation of treatment. It is good practice to routinely collect and analyze CSF obtained before conducting myelograms.

Contraindications • Obtaining CSF in patients with increased intracranial pressure or tentorial herniation (i.e., altered consciousness, head pressing, anisocoria) may precipitate or aggravate tentorial herniation. In such patients, CT or MRI should be performed first, if possible. If CT and MRI are not available, such patients should be premedicated with dexamethasone (0.25 mg/kg) to decrease the CSF production, followed by isoflurane anesthesia with hyperventilation to lower PCO_2. Intravenous (IV) mannitol (0.1 to 0.25 g/kg over 20 minutes) may be given to decrease intracranial pressure. These practices minimize risk. If available, monitoring blood gases ensures that PCO_2 is decreased. Dexamethasone given 10 to 20 minutes before anesthesia does not alter the CSF cell counts, cytology, or protein concentration.

Technique for Collection • CSF is collected from the cerebellomedullary cistern because there tends to be less blood contamination

than with lumbar puncture. The animal is anesthetized via gas anesthetic and positioned in right lateral recumbence for a right-handed clinician or left lateral recumbence for a left-handed clinician. The patient's head and neck are brought to the edge of the table (to allow free hand motion for the clinician during collection), and the head and neck are moderately flexed toward the animal's chest. An assistant is required to firmly hold the patient's head and neck. The point of insertion for the needle is at the crossing of two imaginary lines, one connecting the anterior border of each wing of the atlas and the other drawn from the occipital crest to meet the first line at a 90-degree angle. A 20- to 22-gauge 1½-inch spinal needle is used. The needle is inserted perpendicular to the skin. The patient's head supports the lateral side of the hand used to insert the needle so that the insertion is carefully controlled. When the subarachnoid space is entered, a sudden decrease in the resistance to the needle is felt. During the procedure, the stylet may be removed several times to ascertain if this space has been entered. Once the subarachnoid space has been entered, the collection is done by free flow into a red-top tube, and the sample (1 ml) is submitted for analysis. A red-top tube is preferred over an ethylenediaminetetraacetic acid (EDTA) tube because coagulation factors usually are not found in the CSF, and EDTA increases the measured protein concentration while diluting a sample that is already low in cells.

Difficulties Related to the Collection • These are rare. If the spinal needle is inserted into the spinal cord or caudal brain stem, mild to severe neurologic abnormalities (including death) may result. In mild cases, the animal recovers from anesthesia with body curvature, tetraparesis, and ataxia that are more pronounced on the side of the lesion. In severe cases, the animal has to be ventilated after the collection and may be severely tetraparetic, unable to get up or to lift its head during recovery. In a few patients, unassisted breathing does not return.

Practicing collection shortly after death in dogs and cats selected for euthanasia is a way of gaining experience. Because of reflux of blood into the cranial vault after heart arrest, the CSF pressure is maintained for many minutes after death. If a clinician does not collect CSF and analyze it a few times each month, the procedure is better left to a referral center where it is routinely performed.

Analysis • Routine CSF evaluation includes appearance, red blood cell (RBC) and nucleated cell counts, cytology, and protein concentration. If the volume is limited, a cell count and cytologic evaluation are the first priorities. Appearance, CSF cell counts, cellular sedimentation, and a protein estimate all can be performed in house if necessary.

Appearance • Normal CSF is colorless and clear. Discoloration corresponds to the number of RBCs or nucleated cells present. A visible discoloration is evident when at least 700 RBCs/µl or 200 nucleated cells/µl are present (Rand et al, 1990a). The most common source of RBCs is blood contamination during collection. When this occurs, the clinician can often see a swirl of blood surrounded by clear CSF during collection, whereas hemorrhagic CSF is bloody throughout collection. Centrifugation of the sample helps to differentiate between blood contamination and prior hemorrhage.

Discoloration that persists after centrifugation suggests previous hemorrhage, whereas iatrogenic blood contamination has a clear supernatant. Centrifugation should be performed after cell counts and cytocentrifuged preparations or sedimentation have been completed because centrifugation destroys the cells. Anticoagulants are usually unnecessary because CSF does not normally have coagulation factors. If the sample appears frankly turbid, an EDTA anticoagulated sample should also be taken for cell counts. Xanthochromia or yellow discoloration of CSF appears within 4 to 6 hours after subarachnoid hemorrhage and persists for 3 to 4 weeks.

Cell Counts • The CSF cell counts are performed with a glass hemocytometer chamber. Each side of the hemocytometer chamber is charged with one drop of fresh, undiluted, unstained CSF. After the cells are allowed to settle for 10 minutes, all the cells on both sides of the chamber are counted. The average of the two sides is calculated and then multiplied by 1.1 to produce the number of cells $\times 10^6$/L of CSF (see Figure 2-2). It is imperative to count RBCs and nucleated cells separately so that blood contamination can be evaluated.

Cytology • The CSF nucleated cell count is normally very low; therefore, it is necessary to

concentrate the cells. Centrifugation destroys the cells. Two concentration techniques are commonly used: (1) the cytocentrifugation technique for commercial laboratories and (2) the sedimentation technique for in-house use. Cytocentrifugation requires relatively expensive equipment,* yields an average cell retrieval of approximately 15%, and maintains excellent cellular morphology. It is important to fill the cytocentrifuge chambers carefully, using the same amount of CSF each time so that an estimate of cellular retrieval for quality control is possible.

The sedimentation preparation is inexpensive and yields a cell retrieval of approximately 25% (Jamison and Lumsden, 1988), but the cellular morphology is not as good as with cytocentrifugation. The apparatus consists of a plastic cylinder** of 15 mm in diameter fixed to a glass slide with petroleum jelly; 0.5 ml of CSF is pipetted into the chamber (Figure 14-1), and the cells are allowed to sediment for 30 minutes. The supernatant is then aspirated with a Pasteur's pipette, the cylinder removed, the edge of the remaining fluid gently blotted while the slide is tilted slightly, and the adherent cells quickly air dried via vigorous waving of the slide. Rapid drying of the slide and protection from heat and moisture are essential for preservation of cellular morphology. The cytology is performed in house if the expertise is available. If not, the unstained slide is sent to an experienced clinical pathologist for differential cell count. The sedimentation technique provides rapid cellular evaluation in emergency situations, but whenever possible the CSF or leftover CSF should be sent to a reputable laboratory for cytocentrifuged preparations every time the sedimentation leads to equivocal or unsatisfactory results.

Protein Concentration • Protein concentration can be estimated at the time of collection with a urinary dipstick.† The readings are negative, trace, 30 mg/dl, 100 mg/dl, and 500 mg/dl. The results are reliable below 30 mg/dl and at 100 mg/dl or more. When the results fall around 30 mg/dl, mild to moderate increases (33 to 60 mg/dl) in protein concentration may be missed and read as normal (Jacobs et al, 1990). This estimate should be followed by a quantitative analysis performed at a reputable laboratory.

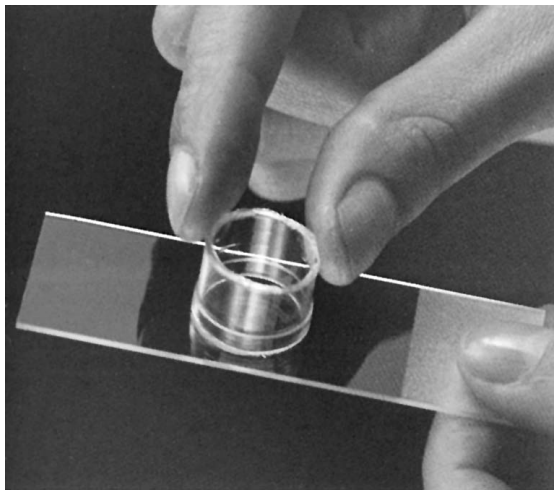

FIGURE 14-1. The well is created with the end of a culturette tube. The smooth end of the cylinder is attached to the microscope slide with petroleum jelly, and the cerebrospinal fluid (CSF) is placed in the well to allow sedimentation of the cells so that cytologic evaluation can be performed.

Normal Values • The reference ranges for canine and feline CSF are presented in Table 14-1. The laboratory report should include gross appearance of the fluid, RBC and nucleated cell counts, total number of cells counted on the slide preparation, differential cell count, and protein quantification. The clinical pathologist should include an absolute count of the cells present on the

TABLE 14-1. Reference Ranges for Feline and Canine Cerebrospinal Fluid

	FELINE*	CANINE†
WBCs ($\times 10^6$/L)	≤2	≤3
RBCs ($\times 10^6$/L)	≤30	≤30
Cytology (%)		
Monocytoid cells*	69-100	87
Lymphocytes*	0-27	4
Neutrophils*	0-9	3
Eosinophils*	0	0
Macrophages (large foamy mononuclear cells)*	0-3	6
Protein (mg/dl)*	≤36	≤33

*Data from Rand JS et al (1990a,b) 1990a.
†Data from Jamison EM (1992a).
RBCs, Red blood cells; *WBCs,* white blood cells.

*Shandon-Elliot Cytocentrifuge, Southern Instruments Ltd, Surrey, England.
**Culturette tube, Fisher Scientific, Don Mills, ONT, Canada.
†Chemstrip 9, Boehringer Mannheim Ltd, Laval, PQ, Canada.

slide preparation. For example, a cell count of 75% neutrophils is meaningless if only four cells were present on the slide preparation. Both concentration techniques cause loss of a large number of cells (cytocentrifuge causing twice as much loss as sedimentation). Volume permitting, two cytocentrifuge preparations are usually requested from the same CSF sample, and the evaluations of the two preparations are combined. With normal cell counts, an absolute nucleated cell count of a few to 50 cells is to be expected on two cytocentrifuge preparations (using 200 µl of CSF per preparation). With nucleated cell counts of greater than or equal to two cells $\times 10^6$/L (i.e., 2 cells/µl), more than 50 cells should consistently be present on the cytology slides. Cytology should always be performed, even if the white blood cell (WBC) count is normal. Contrary to what is often listed as being within reference range by commercial laboratories, a total white cell count greater than 3 cells $\times 10^6$/L in the dog and greater than 2 cells $\times 10^6$/L in the cat is abnormal.

Difficulties Related to Analysis • Whenever possible the CSF sample should be analyzed within 1 hour of collection at a laboratory experienced in handling CSF. Some laboratories do not have stringent procedures for handling CSF (e.g., they do not perform cytology if the nucleated cell count is normal even though the cytology may be abnormal). Although total cell count and protein concentration are stable for up to 48 hours storage at 4° C, the major drawback with CSF remains cell preservation. For remote sites or for samples collected during nonoperational hours of laboratory services, the addition of autologous serum to the CSF preserves cells for up to 48 hours. In these instances the CSF is aliquoted into two tubes: one tube containing nonpreserved CSF (500 µl or 0.5 ml) for measurement of protein concentration and total cell count, and a second tube to which clear serum of the animal's own blood is added to make a concentration of 10% (Bienzle, McDonnell, and Stanton, 2000). The cytologic slides are prepared from the aliquot containing serum. A 500 µl or 0.5 ml CSF sample is enough for two cytocentrifuged preparations. Both tubes are sent to the laboratory.

Effects of Drug Therapies • Corticosteroids reduce nucleated cell count, neutrophil and lymphocyte counts, and protein concentration (Jamison, 1992b), regardless of underlying disease. Therefore CSF analysis should be performed before steroid treatment (except as noted earlier under Contraindications).

Blood Contamination • Blood contamination must be considered during interpretation because it may significantly alter other measured CSF variables. A RBC count greater than 30×10^6/L represents contamination with blood. In cats this RBC count is correlated with the nucleated cell count and the neutrophil percentage (Rand et al, 1990a). An increase of one leukocyte for every 100 RBCs was observed with RBC counts of 30 to 1700×10^6/L. With these counts, 0% to 67% neutrophils were present on cytology. The eosinophil percentage increased from 0% to 15% on these same samples. When the blood contamination is greater than 1700 cells $\times 10^6$/L (i.e., >1700/µl), the WBC count and cytology results are unreliable. The effect of blood contamination on total CSF protein concentration is not as profound. Blood contamination up to 1700 RBCs/µl does not increase the total protein concentration beyond the reference range (Rand et al, 1990b). Blood contamination must also be taken into account when evaluating CSF titers and polymerase chain reaction (PCR) results.

Cerebrospinal Fluid Interpretation • Table 14-2 summarizes expected CSF analysis results for specific disease entities. The CSF results must be interpreted based on history, signalment, duration of signs, and location of the disease (e.g., brain versus spine, parenchyma versus meninges). If there are physical examination, CBC, or serum chemistry abnormalities, the disorder is likely multisystemic, and investigating these abnormalities may provide the diagnosis (e.g., thrombocytopenia associated with a CNS disorder may be caused by *Ehrlichia canis* [see Chapter 15]; nonregenerative anemia in a young dog with a poor appetite and neurologic signs suggests an infectious disorder); appropriate titers may be considered (see the discussion of canine distemper in Chapter 15). If the physical examination and blood studies are normal, the disease is probably limited to the CNS. Because CSF is contained between meningeal layers, CSF analysis is more rewarding when the meninges are involved. Deep parenchymal disease may lead to few or no CSF abnormalities, especially if the disease is mild and acute.

TABLE 14-2. Canine and Feline Cerebrospinal Fluid Analysis in Various Conditions

	APPEARANCE	RBCs/μl*	WBCs/μl	PROTEIN (mg/dl)	CYTOLOGY
Degenerative	Clear, colorless	N	N	N to ↑↑	Mononuclear, macrophages
Neoplastic	Clear, colorless	N to ↑	N to ↑↑	N to ↑↑	Rarely see tumor cells; neutrophils and/or eosinophils may be present; neutrophils in meningioma
Inflammatory					
Bacterial	Hazy to cloudy	N to ↑↑	↑↑ to ↑↑↑	↑↑ to ↑↑↑	Neutrophils early, mixed population later
Viral	Clear, colorless	N	N to ↑	N to ↑	Mononuclear
Feline infectious peritonitis	Hazy to cloudy	N to ↑↑↑	↑↑ to ↑↑↑	↑↑ to ↑↑↑	Mixed
Fungal	Clear to cloudy	N to ↑↑	↑ to ↑↑↑	↑ to ↑↑↑	Neutrophils early, mixed later; organism seen in cryptococcosis; possible eosinophilic pleocytosis
Protozoal (*Neospora*)	Clear	N to ↑	↑ to ↑↑	N to ↑	Mixed with eosinophils
Parasitic	Clear to xanthochromic	N to ↑↑	↑↑ to ↑↑↑	↑↑ to ↑↑↑	Mixed with low % eosinophils
Rickettsial	Clear, colorless	N to ↑	N to ↑↑	N to ↑↑	Mononuclear; neutrophils with Rocky Mountain spotted fever
Granulomatous meningoencephalitis	Clear to cloudy	N to ↑↑	↑ to ↑↑↑	↑ to ↑↑↑	Mixed
Ischemic	Clear, colorless	N	N to ↑	N to ↑	Neutrophils early; mononuclear, macrophage later

*Red blood cell (RBC) count must always be considered in light of possible iatrogenic contamination.
N, Normal; ↑, slight increase; ↑↑, moderate increase; ↑↑↑, marked increase; Mixed, mixed population of mononuclear, lymphocyte, macrophage, and polymorphonuclear cells.

Neutrophils are the first cells on site in an inflammatory response. The more neutrophils present, the more active the inflammatory process. Neutrophils are present in the CSF in many infectious and many idiopathic inflammatory diseases of the dog. In idiopathic (immune-mediated?) CNS disease, the cytologic appearance can be primarily neutrophilic. Before diagnosing steroid-responsive CNS disease (especially in dogs younger than 2 years and in the elderly), infectious disorders must be eliminated. Infectious disease causes the patient to steadily deteriorate; in addition, evidence of infection might be noted on the physical examination (e.g., fever) or blood tests. Neutrophilic pleocytosis may be present in canine meningiomas.

Small lymphocytes represent an antigenic response. The higher the proportion of small lymphocytes, the more likely the syndrome is to respond to steroids. Macrophages are "cleaning up" cells. A predominance of macrophages denotes a degenerative disease (e.g., cellular storage diseases, post-traumatic head injury, encephalopathy). A mixed population of neutrophils, lymphocytes, macrophages, and other mononuclear cells suggests a chronic active inflammatory disease (e.g., granulomatous meningoencephalomyelitis [GME], feline infectious peritonitis, and *Neospora caninum*). Eosinophils are usually absent from normal CSF. A primarily eosinophilic pleocytosis is probably the result of an allergic syndrome, whereas lesser numbers may indicate neoplasia, parasitic infiltration, or fungal diseases (Smith-Maxie et al, 1989).

In the majority of animals with CNS cryptococcosis, encapsulated yeasts are identified on the CSF cytologic preparation (see Figure 11-4 and Color Plate 4E). Neoplastic cells are rare in primary CNS neoplasms, but malignant lymphocytes may be observed in dogs and in cats with CNS lymphoma.

Because albumin and most protein in CSF originate from blood, protein increases in CSF usually indicate a blood-brain barrier breakdown. Local synthesis of immunoglobulins may occur.

Other Measured Cerebrospinal Fluid Variables • Measurements of glucose, lactate dehydrogenase (LD), creatine kinase (CK), and aspartate transaminase (AST) in the CSF have limited usefulness (Rand et al, 1990b). Until specific CNS isoenzymes can be measured, CSF is better used for routine tests. Measurement of intracranial pressure also has limited value. The immunoglobulin G (IgG) index is

$$\frac{\text{CSF [IgG]}}{\text{serum [IgG]}} \times \frac{\text{serum [albumin]}}{\text{CSF [albumin]}}$$

This test enables clinicians to differentiate transudation of serum protein into CSF versus local synthesis of immunoglobulins. The IgG index may identify inflammatory disease even in the absence of CSF pleocytosis (Tipold, 1995; Tipold, Pfister, and Vandevelde, 1993). Although enzyme-linked immunosorbent assay (ELISA) techniques are readily available, only a few laboratories offer the IgG index. Bacterial culture of the CSF is rarely rewarding. The CSF would be better used for measurement of the routine parameters. CSF titers and PCR testing have become readily available for multiple specific diseases. Serum testing should always be done concomitantly and the CSF results evaluated in light of the blood contamination present.

NEUROIMAGING

Frequent Indications • For most practices, spinal radiographs (survey and myelography) are the most useful diagnostic studies for diseases of the vertebral column and spinal cord, whereas CT and MRI are most useful for diseases of the brain and of the middle and inner ear. With the increasing availability of MRI, this is likely to change. Bullae radiographs may help diagnose middle and inner ear diseases, but they have poor sensitivity and specificity. CT and MRI are preferable. Although skull radiographs rarely lead to a diagnosis, they are advised when CT and MRI are unavailable; this is because they are relatively inexpensive and may detect bony skull involvement, intracranial dense space-occupying lesions, distortion of the cerebellar tentorium, or a combination thereof.

Technique • The quality of the radiographs, adequate preparation of the animal, and the position of the animal are important. Neuroradiography should be undertaken with the animal under general anesthesia. It is a waste of time and money to attempt spinal radiographs of an animal with manual restraint or sedation alone, especially if pain is present (e.g., in intervertebral disk disease, relaxation provided by sedation is never adequate to relieve muscle spasms present at the diseased site). Position of the animal is also crucial. The animal must be in perfect lateral recumbence; the ribs should be superposed. The vertebral column must be parallel to the table throughout its length. The checkpoint is to make sure that the ends of the vertebrae are not visible; if they are, it denotes a curvature in the dog's body. Foam pads are necessary to produce a perfectly straight spine.

In the cervical area, the C4-C5 intervertebral space is often erroneously read as abnormal. A dog's head and thorax are of different sizes, and if appropriate padding is not used when the dog is in lateral recumbence, an upward concavity of the spine results. Two lateral views should be taken: (1) upper and (2) lower cervical. For the ventrodorsal view, the animal should be extubated. For thoracolumbar (T3-L3) studies, three lateral views are necessary: (1) thoracic, (2) thoracolumbar, and (3) lumbar. For lumbosacral studies, a cone-down of the area is performed. The ventrodorsal views are matched with the lateral views. The same views are repeated for the myelographic study with a cone-down of the suspicious area. In most cases of thoracolumbar disk disease, the diseased site is observable on survey radiographs. The myelogram provides information on the extent of spinal cord involvement and on the lateralization of the lesion.

Only experienced clinicians should perform myelography. The study should be done in the facility where the animal is possibly scheduled for neurosurgery. The contrast injections are done at the L5-L6 intervertebral space. Cervical injections may be used for cervical lesions. Poor technique and positioning are the most common causes of misdiagnosis in spinal column diseases.

Bullae series are seldom rewarding in diagnosis of middle and inner ear diseases; only advanced or severe changes can be observed on radiographs. Destructive bony lesions (i.e., infectious, neoplastic) of the tympanic bullae and narrowing of the external canals with and without calcifications are obvious.

Usually only a subtle difference in density between tympanic bullae is noted. Two oblique views and an open-mouth view are the most useful. Extubation should also be performed for the dorsoventral view of the skull. If an intracranial space-occupying lesion is suspected, two lateral views of the skull are advised: (1) right sided and (2) left sided.

MUSCULAR WEAKNESS

Weakness may be the result of neuromuscular disease, as well as metabolic disorders, cardiopulmonary disorders, or both. A partial listing of some of the more common causes is given in Table 14-3. History, physical examination, electrocardiogram (ECG), CBC, serum chemistries (e.g., CK, lactic acid), electrodiagnostics, and selected serologic tests (i.e., antibodies to acetylcholine receptors) usually allow diagnosis. See discussions on blood glucose, serum calcium (see Chapter 8), and potassium (see Chapter 6) for additional information.

Electrodiagnostics

Infrequent Indications • The diagnostic workup of myopathies, neuropathies, nerve sheath tumors, myasthenia gravis, and cauda equina syndrome is enhanced by electromyography, nerve conduction velocities, and repetitive nerve stimulations. Electromyography is the most widely used of these tests and probably the most useful. Electromyography may allow lesion localization; in other cases, it allows evaluation of function versus the anatomopathologic evaluation obtained from muscle and nerve biopsies.

Brain stem auditory evoked responses (BAER), visual evoked potentials, and somatosensory evoked potentials assess ascending pathways. BAER is the most useful diagnostically. It is used as a screening test for hearing in animals suspected of having congenital nerve deafness and aids in diagnosis of vestibular syndromes. Whenever possible, BAER is done in all dogs and cats with head tilt. By evaluating the cochlear part of the vestibulocochlear nerve, BAER allows diagnosis of idiopathic peripheral vestibular disorders in which only the vestibular part is affected. BAER also evaluates the brain stem. It is particularly helpful in evaluating response to treatment in animals that previously underwent BAER.

Electroencephalography has been poorly used in veterinary medicine; its usefulness has been controversial. It should be performed without anesthesia.

TABLE 14-3. Selected Causes of Muscular Weakness in the Dog and Cat

Hypoglycemia
Hypokalemia
Hyperkalemia
Hypercalcemia
Hypoxia
Anemia
Pulmonary disease
Pulmonary thromboembolism
Cardiac disease causing decreased cardiac output
Primary Myopathies
Inflammatory
Degenerative (e.g., muscular dystrophy)/metabolic
Junctionopathies (i.e., myasthenia gravis)
Neuropathies
Primary neuropathy (e.g., polyradiculoneuritis, botulism)
Secondary neuropathy
Hypothyroidism
Paraneoplastic neuropathy
Diabetes mellitus

Creatine Kinase

Infrequent Indications • Suspected muscle disorder (e.g., patients with generalized weakness but no ataxia). The weakness is displayed by a stiff, short-strided gait. The spinal reflexes in most ambulating cases are adequate but may be significantly decreased if the muscle disorder is severe. In slowly progressive cases, generalized muscle wasting may be the only abnormality.

Advantage • The test is specific for skeletal and cardiac muscle disease.

Disadvantage • It is an extremely sensitive indicator of muscle damage and thus is often elevated by muscle damage secondary to other problems (i.e., recumbent animal with pressure damage, seizure activity).

Analysis • Storage of serum sample at any temperature results in a loss of CK activity. The method of choice for analysis adds a reducing agent to the incubation medium to reactivate CK activities lost during storage. The normal values are "activated" values. If serum is not assayed immediately, it should be refrigerated. Exposure to bright light should be avoided. If samples are to be shipped or stored for longer than 2 days, they should be frozen.

Normal Values • As with other enzymes, these vary among laboratories, depending on technique and units used. At the author's laboratory, the reference range is less than 460 IU/L for dogs and less than 580 IU/L for cats.

Danger Values • None.

Artifacts • See Introduction to Serum Chemistries.

Causes of Increased Creatine Kinase • Many laboratories include CK in their standard biochemical profile; therefore, more problems are associated with interpretation of an increased CK than with inappropriate request of the test. CK is a sensitive indicator of muscle damage and only large increases (≥ 10,000 IU/L) or persistent increases, even if moderate (> 2000 IU/L), generally are of clinical significance. Significant muscle disease may exist without CK elevation. If degenerative pathology predominates, CK may be normal or only mildly elevated. Mild increases are observed with restraint, physical activity, intramuscular (IM) injections, prolonged recumbency, and muscle biopsies. Moderate increases occur with trauma, convulsions, continuous trembling or shivering, and some neuropathies and myopathies. Large increases are primarily caused by myositis.

Feline obstructive urethral syndrome (CK is present in the urinary bladder) and muscle ischemia secondary to status epilepticus are occasionally observed with CK greater than 10,000 IU/L. Because the half-life of CK is short, the test should always be repeated to determine if increased activity persists. Persistent elevations strongly suggest active muscle damage and indicate electromyography and muscle and nerve biopsies. Histochemical analysis of muscle biopsy specimens is useful in defining myopathies. The laboratory processing the biopsies should be contacted ahead of time and their protocol for storing and shipping the samples adhered to rigidly.

Myositis and myopathy are uncommon but can have the following origins: infectious (e.g., *N. caninum, Toxoplasma gondii*), immune-mediated (e.g., masticatory myositis, polymyositis), toxic (e.g., monensin poisoning), endocrine (e.g., hypothyroidism, hyperadrenocorticism), congenital (e.g., muscular dystrophy), nutritional (e.g., vitamin E or selenium), or exertional (e.g., exertional myopathy).

Lactic Acid

Infrequent Indications • Suspected metabolic myopathies, especially in Labrador retrievers; evaluation of metabolic acidosis; evaluating perfusion in patients being treated for shock; helping determine prognosis for critically ill animals (e.g., dogs with gastric dilatation/volvulus).

Disadvantage • Sample handling is very important.

Analysis • Lactate is assayed in lithium heparin plasma or from blood collected in iodoacetate tubes. Fluoride tubes may be similar. Analysis may be performed with standard laboratory analyzers or with point-of-care instruments.

Normal Values • 2 to 13 mg/dl (0.22 to 1.44 mmol/L)

Danger Values • Values greater than 6.0 mmol/L are associated with a poor prognosis (see following).

Artifacts • Venous stasis (e.g., prolonged holding off of the vein), struggling during venipuncture, and a recent meal may significantly increase blood lactate levels. Samples taken from cephalic veins tend to have slightly higher values than samples taken from jugular veins. The plasma should be quickly separated from the RBCs or the blood stored at 4°C for less than 2 hours before plasma is harvested. If the blood sample is allowed to sit at room temperature, the lactate concentration progressively increases. If iodoacetate tubes are used, blood can be held at room temperature for up to 2 hours before harvesting plasma. Harvested plasma should be refrigerated or frozen. Oxaloacetate anticoagulant may cause artifacts. Aspirin, epinephrine, and phenobarbital may alter blood lactate levels.

Causes of Increased Lactic Acid • One reason for measuring lactic acid levels in dogs is to look for metabolic myopathies in Labrador retrievers (Shelton, 1993). The first blood sample is taken with the dog at rest. Then the dog is exercised by walking it briskly for 10 to 15 minutes, and another blood sample is taken. A marked increase above the normal range after minor exercise suggests this disorder. Another reason is to evaluate

patients (usually critically ill) for lactic acidosis (Lagutchik et al, 1997) and to help determine their prognosis. Dogs with gastric dilatation/volvulus that have blood lactate concentrations greater than 6.0 mmol/L have a worse prognosis and are more likely to have gastric wall necrosis (De Papp, Drobatz, and Hughes, 1999). Persistent increased lactic acid concentrations in animals being treated for shock indicates that adequate perfusion is probably not being achieved and appears to be associated with a poorer prognosis.

Acetylcholine Receptor Antibody

Occasional Indications • Myasthenia gravis may be demonstrated by any of the following: weakness that may or not worsen with exercise, acquired megaesophagus with or without concomitant pharyngeal and laryngeal weakness, weak palpebral reflexes, pharyngeal and laryngeal weakness, or a combination thereof. Three clinical forms have been recognized: (1) focal (no appendicular weakness), (2) fulminating (appendicular weakness with acute onset and rapid development), and (3) generalized (appendicular weakness but without acute onset) myasthenia (Dewey et al, 1997). Contrary to what was previously described, exercise-associated weakness is not *always* a prominent historical or clinical feature.

Advantage • The test is specific for myasthenia gravis.

Disadvantage • Some dogs with myasthenia gravis are seronegative for antibodies against acetylcholine receptors (AchRs). When immunocytochemical assays are used in these dogs, immune complexes may be detected at neuromuscular junctions, indicating that antigens other than AchR are present (Shelton et al, 1990). Repetitive nerve stimulation studies may be diagnostic in seronegative cases, even those with focal myasthenia (Dewey et al, 1997; Shelton et al, 1990).

Analysis • The test requires 1 ml of serum. The sample is sent on dry ice by an overnight carrier (see Appendix I for availability).

Normal Values • Less than 0.6 nM/L. No correlation exists between AchR antibody concentration and severity or distribution of the clinical signs (Dewey et al, 1997).

Danger Values • None.

Drug Therapy That May Alter Results • In principle, corticosteroids and other immunosuppressive drugs may lead to false-negative results.

Cause of Increased Titers • Myasthenia gravis.

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15

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Microbiology and Infectious Disease

Infectious agents may be identified directly by cytologic analysis, histopathologic evaluation, culture, viral isolation, antigen detection, or polymerase chain reaction (PCR). Detection of antibodies against infectious agents provides indirect evidence of prior exposure or current infection. This chapter describes methods for obtaining specimens, outlines currently used testing procedures for the more common infectious diseases, and discusses interpretation of results from the various procedures and tests.

WHEN TO SUSPECT BACTERIAL/ FUNGAL/RICKETTSIAL VIRAL AGENTS

Infectious diseases should be on the differential list for most problems, especially those with fever or signs of inflammation. History, physical examination findings, and routine clinical pathologic testing are seldom pathognomonic for an infectious cause, but they help the clinician rank differential diagnoses and develop a logical diagnostic plan.

Historical findings can increase the degree of suspicion for infectious diseases. Exposure to other infected animals or contaminated fomites is important for agents with direct transmission, such as those inducing respiratory disease (e.g., feline herpesvirus 1, canine bordetellosis) or gastroenteritis (e.g., canine and feline giardiasis, canine and feline parvovirus infection). Potential exposure to vectors (e.g., mosquitoes for dirofilariasis; ticks for Lyme borreliosis [*Ixodes* spp.], ehrlichiosis *[Rhipicephalus sanguineus]*, Rocky Mountain spotted fever [RMSF; *Dermacentor* spp.], babesiosis *[R. sanguineus]*) or appropriate travel history (e.g., coccidioidomycosis in the Southwest; RMSF in the Southeast; blastomycosis in the Mississippi, Missouri, and Ohio River valleys) can also suggest an infectious disease. Vaccination history, deworming history, and determination of whether other animals or people in the environment are also affected can aid in ranking infectious diseases on a differential diagnoses list.

Physical examination findings may suggest an infectious cause. Infectious agents can induce fever. Lymphadenomegaly as a result of reactive lymphoid hyperplasia can be infectious in origin. Hepatosplenomegaly can be caused by immunologic stimulation induced by chronic intracellular infections (e.g., ehrlichiosis, brucellosis). Endogenous uveitis commonly occurs after infections by feline immunodeficiency virus (FIV), feline infectious peritonitis (FIP) virus, toxoplasmosis, and systemic mycoses. Mucopurulent discharges can suggest primary or secondary bacterial infections. Certain infectious diseases cause specific abnormalities such as dendritic ulcers (feline herpesvirus 1), chorea mycolonus (canine distemper virus), or testicular swelling plus pain (canine brucellosis).

Finally, clinicopathologic abnormalities can suggest disease caused by infectious agents. Neutrophilic leukocytosis, particularly if found concurrently with a left shift or degenerative neutrophils (see Chapter 4), is consistent with an infectious cause of disease. Gram-negative sepsis is suggested by leukopenia with a degenerative left shift. Monocytosis can be induced by persistent infection with a number of intracellular agents that result in persistent infection. Polyclonal (e.g., multiple infectious causes) or monoclonal gammopathies (e.g., usually induced by neoplasia, rarely associated with canine ehrlichiosis) may suggest chronic immune stimulation. Neutrophils in aqueous humor, cerebrospinal fluid (CSF), synovial fluid, or urine may indicate inflammation induced by infectious agents.

CYTOLOGY

Common Indications • Cytologic examination of exudates, blood film, tissue imprint, aspiration biopsy, or wet mount of hair is indicated when bacterial and fungal diseases (and occasionally rickettsial and viral diseases) are suspected.

Advantages • Inexpensive, readily available, and may allow rapid confirmation and identification of an infectious agent. Assists in establishing normal flora and contaminants versus infection (e.g., interpretation of relative numbers of bacteria and yeasts in the ear canal). Gives visualization of relative numbers of organisms at time of collection (culture results may be misleading in terms of fast- or slow-growing bacteria).

Disadvantages • Infectious agents cannot always be found (e.g., ehrlichiosis, haemobartonellosis, infections with numbers of organism that are below sensitivity level of cytology). Sometimes a presumptive cytologic diagnosis must be confirmed by other methods (e.g., histopathology, culture), and cytology is of limited value in detecting viral inclusions except in brief viremic stages of canine distemper.

Specimen Procurement and Analysis

See Chapter 16 for discussion of cytologic techniques and cytologic conclusions.

Bacterial Diseases

Discharges from animals with suspected bacterial disease should be placed on a microscope slide, air dried, fixed, and stained with both Gram's and Romanowsky's-type

stains (see Chapter 16). The examination is started on low power (10x magnification), with oil immersion (100x) used for inspection of bacterial morphologic features (i.e., rods, cocci) and Gram's stain characteristics (i.e., gram-positive [blue] or gram-negative [pink]). The primary disadvantage of Gram's staining is that gram-negative bacteria may be difficult to find because background material stains pink. It is easier to find bacteria (dark-blue stain) and easier to study morphologic detail of other cells (i.e., inflammatory cells) using Romanowsky's-type stains. Gram's staining may be variable; organisms in body fluids may stain differently from those grown on a blood agar plate. Gram's stain demonstrates the gram-positive, branching filaments of *Actinomyces* spp. and *Nocardia* spp. (see Color Plate 4C). Acid-fast stains can be used for *Mycobacterium* spp. and to help differentiate *Nocardia* spp. (acid-fast) from *Actinomyces* spp.

Some bacteria have characteristic morphologic features. Large rod-form bacteria containing spores found on fecal cytology of dogs or cats with diarrhea suggest *Clostridium perfringens* (Color Plate 4F; see Chapter 9). Bipolar-staining, gram-negative coccobacilli found in aspirates of inflamed cervical lymph nodes from cats in the Southwest or West suggest *Yersinia pestis*. Short spirochetes found on fecal cytology of animals with diarrhea suggest campylobacteriosis. Spirochetes found on cytology of gastric mucosa of vomiting animals suggest helicobacteriosis.

To demonstrate inclusion bodies in acute feline chlamydial conjunctivitis, conjunctival scrapings are obtained with a flat spatula, smeared on a slide, stained with Romanowsky's-type stains, and examined for intracytoplasmic aggregations of *Chlamydophila felis* (previously *Chlamydia*).

Morulae of *Ehrlichia* spp. are rare in the cytoplasm of mononuclear cells *(Ehrlichia canis)*, neutrophils *(Ehrlichia ewingii*; *Anaplasma phagocytophila* [previously *E. equi*]), or platelets *(Ehrlichia platys)*. *Mycoplasma haemofelis* (cats only), *M. haemominutum* (cats only), *Haemobartonella canis*, *Cytauxzoon felis* (cats only), and *Babesia* spp. sometimes infect canine or feline erythrocytes.

Cutaneous Parasitic Diseases

For demonstration of *Cheyletiella* spp., a piece of transparent adhesive tape is gently pressed against areas with crusts or dandruff and then placed on a microscope slide. Next the hair is clipped, mineral oil is placed on the skin and on a microscope slide, and the skin is scraped using a blunt No. 10 scalpel blade. For skin scrapings to look for *Demodex* spp., the skin should be immobilized and mites expressed from follicles by pinching and scraping the extruded material. For scrapings to look for *Sarcoptes* spp. or *Cheyletiella* spp., the scraping is continued more superficially (inducing a mild capillary ooze) over a larger surface area. After transfer of the scraping, the microscope slide field is scanned at 10x for mites.

Fungal Diseases

For identification of dermatophytes, hairs are plucked from the periphery of a lesion, placed on a microscope slide, and covered with 10% to 20% potassium hydroxide to clear debris. The slide is then heated (not boiled) and examined under the 10x or 40x objective to search for hyphae, spores, conidia, budding yeasts, and fungus-induced damage (e.g., swollen or broken hair shafts). The 40x objective is used to identify arthrospores (dense aggregates of spherical structures that may cover the hair shaft [see Color Plate 3C]). Failure to find arthrospores does not rule out dermatomycosis. Culture is more sensitive for diagnosis of dermatophytosis (see Fungal Culture).

Romanowsky's-type stains (e.g., Wright's) are used in preference to wet-mount preparations and ink when looking for fungi other than dermatophytes (see Chapter 16). Romanowsky's-type stains are also useful in identifying yeasts such as *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Sporothrix schenckii*, *Coccidioides immitis,* or *Cryptococcus neoformans* (see Color Plate 4E) in exudates, CSF, lymph node aspiration cytology, or transtracheal aspiration cytology.

Viral Diseases

Canine distemper virus inclusions in lymphocytes, neutrophils, or erythrocytes (Color Plates 2D and 2E) are diagnostic of infection but are only present transiently, so false-negative results are common. Rarely, FIP-inducing strains of coronavirus result in transient intracytoplasmic inclusions in circulating neutrophils.

CULTURE AND ANTIMICROBIAL SUSCEPTIBILITY

Common Indications • Culture and antimicrobial susceptibility is indicated in most suspected bacterial diseases (Table 15-1), especially when clinical syndromes have been resistant to medications. *Remember:* Skin and mucosal surfaces have a resident microflora (Table 15-2); therefore, care must be taken to avoid contamination.

Advantages • Usually allows the most effective treatment to be administered.

Disadvantages • Requires time for agents to grow; some organisms are fastidious or have special culture requirements; the expense; and ease of contaminating or making inactivate cultures, rendering results worthless.

Bacterial Culture

Specimen Procurement

Body Cavities • The site of skin puncture should be prepared as for blood culture (see discussion in following section). If pyothorax or peritonitis seems likely but fluid cannot be aspirated, lavage (see Chapter 10) is indicated. Because mixed infections are common and pure anaerobic infections may occur, aerobic and anaerobic cultures should be performed.

Cardiovascular System • Blood cultures are indicated in suspected bacterial endocarditis or septicemia. A large vein prepared surgically with sequential iodine and alcohol scrubs is used for three blood culture specimens obtained during a febrile episode over a 24-hour period in dogs with suspected endocarditis. Culture of fewer than three specimens significantly decreases the chance of positive results. At least 5 ml of blood is placed directly into transport media* that will support the growth of aerobic and anaerobic bacteria, and it is incubated at 20° C for 24 hours. Clotted blood or blood containing ethylenediaminetetraacetic acid (EDTA) or citrate are unacceptable because it decreases isolation of organisms (*Bartonella* spp. are

*BBL Septi-Chec, Becton Dickinson Microbiology Systems, Sparkes, Md.

TABLE 15-1. Bacteria Commonly Isolated from Various Sites in Infectious Disorders in Dogs and Cats

Integument
Pyoderma
- *Staphylococcus aureus/intermedius*
- *Proteus* spp.
- *Pseudomonas* spp.
- *Escherichia coli* (usually secondary to staphylococci)

Ear
- *Malassezia* spp.
- *Pseudomonas* spp.
- *S. aureus/intermedius*
- *Proteus* spp.

Respiratory System
Pneumonia
- *Pseudomonas* spp.
- *E. coli*
- *Klebsiella* spp.
- *Pasteurella* spp.
- *Bordetella* spp.
- *Staphylococcus* spp.
- *Streptococcus* spp.
- *Mycoplasma* spp.

Pleural Cavity
- *Nocardia* spp.
- *Actinomyces* spp.
- *Pasteurella* spp.
- Anaerobes

Gastrointestinal (GI) Tract
Intestine
- *Salmonella* spp.
- *Campylobacter* spp.
- *Clostridium perfringens*
- *E. coli*

Genitourinary Tract
- *E. coli*
- *Proteus* spp.
- *Klebsiella* spp.
- *S. aureus/intermedius*

Eye
Conjunctiva and Cornea
- *S. aureus* (coagulase positive and negative)
- *Streptococcus* spp.
- *S. epidermidis*
- *E. coli*
- *Proteus* spp.
- *Bacillus* spp.

Cardiovascular System
Aerobes
- *S. aureus*
- Beta-hemolytic streptococci
- *E. coli*
- *Klebsiella* spp.
- *Pseudomonas* spp.
- *Proteus* spp.
- *Salmonella* spp.

Anaerobes
- *Bacteroides* spp.
- *Fusobacterium* spp.
- *Clostridium* spp.

Data compiled from Greene CE, editor: *Clinical microbiology and infectious diseases of the dog and cat*, Philadelphia, 1998, WB Saunders.

TABLE 15-2. Normal Bacterial Flora at Various Sites in Dogs and Cats

Integument		
Skin		
Aerobes		
	Micrococcus spp.	
	Staphylococcus spp.	
	Streptococcus spp.	
	Gram-negative rods including *Pasteurella* spp.	
	Diphtheroids	
Anaerobes		
	Clostridium spp.	
Ear		
Aerobes		
	Staphylococcus spp.	
	Corynebacterium spp.	
	Streptococcus spp.	
	Coliforms	
	Bacillus spp.	
Yeast		
	Malassezia spp.	
Respiratory System		
Nasal Cavity, Pharynx		
Aerobes		
	Staphylococcus spp.	
	Streptococcus spp.	
	Neisseria spp.	
	Corynebacterium spp.	
	Escherichia coli	
	Lactobacillus spp.	
	Proteus spp.	
Anaerobes		
	Clostridium spp.	
	Bifidobacterium spp.	
	Propionibacterium spp.	
	Fusobacterium spp.	
	Bacteroides spp.	
Trachea		
	Streptococcus spp.	
	Staphylococcus spp.	
	Pasteurella spp.	
	Klebsiella spp.	
	Corynebacterium spp.	
Eyes		
Cornea and Conjunctiva		
Aerobes		
	Staphylococcus spp. (coagulase positive and negative)	
	Nonhemolytic, alpha- and beta-hemolytic streptococci	
	Bacillus spp.	
	Pseudomonas spp.	
	E. coli	
	Corynebacterium spp.	
	Neisseria spp.	
	Moraxella spp.	
Gastrointestinal Tract		
Oral Cavity and Feces		
Aerobic		
Gram +	*Streptococcus* spp.	
	Staphylococcus spp.	
	Bacillus spp.	
	Corynebacterium spp.	
Gram –	Enterobacteriaceae (especially *E. coli*, *Enterobacter* spp., *Proteus* spp., and *Klebsiella* spp.)	
	Pseudomonas spp.	
	Neisseria spp.	
	Moraxella spp.	
Anaerobic		
Gram +	*Clostridium* spp.	
	Lactobacillus spp.	
	Propionibacterium spp.	
	Bifidobacterium spp.	
Gram –	*Bacteroides* spp.	
	Fusobacterium spp.	
	Veillonella spp.	
Other	Spirochetes	
	Mycoplasma spp.	
	Yeasts	
Genitourinary Tract		
Distal Urethra and Prepuce		
Gram +	*S. aureus*	
	S. epidermidis	
	Streptococcus spp.	
	Mycoplasma spp.	
	Bacillus spp.	
	Corynebacterium spp.	
Gram –	*Flavobacterium* spp.	
	Haemophilus spp.	
	Moraxella spp.	
	Pasteurella spp.	
	Klebsiella spp.	

Data compiled from Greene CE, editor: *Clinical microbiology and infectious diseases of the dog and cat*, Philadelphia, 1998, WB Saunders.

exceptions; they can be cultured from EDTA tubes). If a patient is critically ill and sepsis is suspected, three cultures should be obtained over 1 to 3 hours before antimicrobial therapy is instituted. Because the urinary system is a common portal of entry for bacteria into the body, urine is often cultured in patients when the source of septicemia or bacterial endocarditis is unknown.

Central Nervous System • Bacterial infection of the central nervous system (CNS) is uncommon. Even when infection occurs, low numbers of organisms make cytology and culture low-yield procedures. If increased numbers of neutrophils and increased protein are detected in CSF (see Chapter 14), however, aerobic and anaerobic bacterial culture and antimicrobial susceptibility testing are indicated. CSF should be placed in transport media* and delivered to the laboratory as soon as possible. Aerobic and anaerobic bacterial culture should be performed when bacterial infection of the CNS is suspected.

Eye • Conjunctival culture should be performed before topical anesthesia or

*Trypticase Soy Broth, Becton Dickinson Microbiology Systems, Cockeysville, Md.

application of fluorescein stain by rolling a moistened sterile swab over the conjunctiva. Ocular paracentesis is necessary for intraocular culture.

Gastrointestinal Tract • Primary bacterial gastroenteritis occasionally occurs. *Salmonella* spp., *Campylobacter* spp., *C. perfringens*, and *E. coli* are important genera. These organisms can also be isolated from normal animals, however. *Salmonella* spp. and *Campylobacter* spp. can cause small or mixed bowel diarrhea; *C. perfringens* is usually associated with large bowel diarrhea. Approximately 2 to 3 g of fresh feces should be submitted to the laboratory for optimal results. If delayed transport of feces to the laboratory is expected, the clinician should consult the laboratory for appropriate transport media. Because these organisms have special culture requirements, the laboratory must be notified of the suspected pathogen. A positive culture for *C. perfringens* does not prove it was the cause of disease because not all *C. perfringens* produce enterotoxin.

Genitourinary Tract • Urine obtained by cystocentesis is preferred for urine culture. If a patient is severely thrombocytopenic (< 50,000/µl), or if cystocentesis cannot be performed, catheterization or a midstream-voided sample is acceptable (quantitative culture is needed). Isolation of bacteria should always be assessed concurrently with the urine sediment. Rarely, difficult to diagnose urinary tract infections require maceration and culture of a bladder wall biopsy specimen. Calculi should be crushed with a sterile mortar and pestle and cultured. Culture for *Mycoplasma* spp. or *Candida* spp. should be performed if pyuria is identified in absence of calculi, masses, and aerobic bacteria.

Culture of the third fraction of an ejaculate (preferred) or prostatic massage is recommended for prostatic culture. Culture of the second fraction of an ejaculate is recommended for testicular culture. Culture of prostatic or testicular material retrieved by aspiration or biopsy can also be performed. Prostatic massage and closed prostatic aspiration or biopsy should be avoided in dogs with suspected prostatic abscesses. Obtaining distal urethral specimens for quantitative culture before and after ejaculation may help avoid confusion caused by urethral contamination. Anaerobic culture of urine or prostatic fluid is rarely useful.

Integument and Ear • In superficial pyoderma, hair is clipped from the surrounding area, but disinfection is not attempted. A pustule is ruptured with a sterile fine-gauge needle, and a swab of pus is cultured. In deep pyoderma, hair surrounding the lesion is clipped and the area is disinfected with an antiseptic. The lesion is squeezed to express exudate, which is collected on a swab. Gloves should be worn.

For culture of ears, a sterile otoscope cone is inserted to the level of the horizontal canal and the ear is swabbed through the cone. When middle-ear infection is suspected, the animal is anesthetized and material for culture is retrieved by myringotomy by penetration of the tympanum with a sterile CSF needle placed through a sterile otoscope cone.

Musculoskeletal System • No normal flora exists in musculoskeletal tissues. Dogs with radiographic evidence of diskospondylitis should be evaluated for *Brucella canis* infection serologically (see Serologic Tests for Bacterial Infections). Intervertebral joints can be cultured after fluoroscopically guided aspiration or when decompressive spinal surgery is required. Most cases of diskospondylitis develop after hematogenous spread of bacteria from an extravertebral source. Blood and urine are commonly cultured from patients with diskospondylitis; *Staphylococcus* spp. are commonly involved.

Dogs or cats with suppurative arthritis (with or without cytologic visualization of bacteria) should have the synovial fluid cultured for aerobes and *Mycoplasma* spp. (see Chapter 10). Likelihood of positive culture results increases if the synovial fluid contains degenerative neutrophils. L-form bacteria usually cannot be grown from joint fluid via routine culture techniques. Synovial biopsy for culture plus histopathologic evaluation for L-form bacteria is more sensitive than only culture of fluid. *Borrelia burgdorferi* is almost never isolated by routine culture from joints of dogs with Lyme disease.

In osteomyelitis, culture of fistulous tracts is less sensitive than culture of affected bone. Culture for infectious myositis is seldom performed unless suspicion for an anaerobic infection (e.g., *Clostridium* spp.) is based on foul odor, subcutaneous (SC) emphysema, or empyema. The clinician can better evaluate for other infectious myopathies (e.g., toxoplasmosis, leptospirosis) using serologic testing or PCR assays.

Respiratory System • Lower airway specimens are best obtained by transtracheal aspiration or bronchoalveolar lavage during bronchoscopy. Fine-needle pulmonary aspiration biopsy can be used but carries more risk (see Chapter 11). Bacteria can be isolated from the trachea in some clinically healthy dogs. These bacteria are probably transient; common isolates are listed in Table 15-2. Because many organisms isolated from normal dogs have also been associated with lower respiratory tract inflammation, all transtracheal aspiration samples should be evaluated by culture, antimicrobial susceptibility, and cytology. With cytology, the clinician should look for squamous cells coated with bacteria (which indicates oropharyngeal contamination) (see Figure 11-10). Bacteria should not be considered significant unless accompanied by neutrophilic inflammation. *Mycoplasma* spp. have been isolated in pure culture from lower airways of patients with clinical signs of respiratory disease (Randolph et al, 1993; Chandler and Lappin, 2002). Culture for *Mycoplasma* spp. should be performed on all transtracheal aspiration samples; these samples need to be transported to the laboratory in Amies' medium or modified Stuart's bacterial transport medium. *Mycoplasma* spp. culture should be specifically requested.

Nasal specimens are best obtained from nasal lavage, core biopsy, or by passing a swab through a sterile otoscope cone (see Chapter 11). The clinician can best obtain pharyngeal specimens using a guarded swab taken during pharyngoscopy. Nasal and pharyngeal cultures can be difficult to interpret because of extensive normal flora in the nasal cavity and nasopharynx (see Table 15-2).

Specimen Transport

For aerobic culture, no special transport medium is required if the swab remains moist and can be inoculated onto the culture medium within 3 hours. Swabs containing liquid* or gel transport† media are frequently used, however. Routine cultures can be safely stored in transport media at room temperature for up to 4 hours. After this time, overgrowth is a potential problem because of various growth rates of different organisms. Refrigerated, routine specimens can be stored in transport media for at least 2 days. Tissue samples can be refrigerated for up to 2 days. Fluids (e.g., urine) can be safely stored at room temperature for 1 to 2 hours, refrigerated for 24 hours, and refrigerated in transport media for 72 hours (Jones, 1998). Quantitative culture is not accurate for fluids stored in transport media because of artifactual dilution.

For anaerobic culture, fluid should be aspirated into a syringe, the needle capped with a rubber stopper, and the sample inoculated onto culture medium within 10 minutes of collection. Transport media that support the growth of anaerobic bacteria are available† but are not ideal for fastidious *Bacteroides* spp. and *Fusobacterium* spp. With these limitations, samples can be refrigerated for 2 days in an appropriate transport medium.

Analysis • Blood agar plates grow most routine bacterial pathogens. A biplate containing blood agar and MacConkey's agar is frequently used. The common anaerobic culture medium is thioglycolate. The decision to perform in-office testing instead of using a commercial laboratory is based on caseload and available equipment. With the exception of blood and feces, the majority of culture procedures can be performed in office. Readers are referred elsewhere for details of equipment and operation of an in-office microbiology laboratory (Hirsh and Ruehl, 1986).

Sensitivity Testing • Sensitivity testing gives an in vitro estimation of suitability of a given concentration of an antimicrobial agent. Two techniques are used: (1) the dilution test and (2) the disk diffusion test.

Dilution Test • This test is quantitative and determines the least amount of antimicrobial needed to prevent growth of a microorganism (minimum inhibitory concentration [MIC]). Quantitative susceptibility testing is indicated when antimicrobial dosing schedules need to be monitored closely (e.g., gentamicin) or when disk test results are inapplicable, equivocal, or unreliable (e.g., slow-growing organisms, confirmation of susceptibility to polymyxins, confirmation of susceptibility or resistance to given doses of aminoglycosides). Other indications include anaerobes and testing for synergism or antagonism between antimicrobials.

*Culturette, American Scientific Products, McGaw Park, Ill.
†BBL CultureSwab Plus, Becton Dickinson Microbiology Systems, Sparkes, Md.

Advantages • May be effective even though disk diffusion techniques suggest otherwise (e.g., antibiotics concentrated in urine).

Disadvantages • Expense, inability to perform in office, and need to determine if required concentrations of a certain antibiotic are feasible. Ideally, blood concentrations of drugs should be more than four times the MIC and urine concentrations 10 to 20 times the MIC. MIC sensitivity for topically administered antimicrobials is seldom determined because these methods are based on blood or urine concentrations.

Disk Diffusion Test • This is the most widely used method in clinical practice (i.e., Kirby-Bauer technique). A zone of inhibition of bacterial growth is noted around a disk containing a fixed amount of antibiotic. The procedure is qualitative and allocates organisms to the sensitive (susceptible), intermediate (indeterminate), or resistant category.

Advantages • Simplicity and suitability for most routine cultures, can be performed in office, and applicability for rapidly growing organisms (e.g., Enterobacteriaceae, *Staphylococcus aureus*).

Disadvantages • Not suitable for slow-growing organisms and anaerobes; inaccuracy in predicting susceptibility of poorly diffusing antibiotics (e.g., polymyxins); factors that influence the test (e.g., pH and thickness of the medium, concentration of organisms, incubation time) must be standardized. It is imperative that proper procedures be followed to avoid errors in diagnosis.

Artifacts • Artifacts result from improper sample collection (i.e., wrong sample, contamination), improper sample transport, failure to notify the laboratory of suspected pathogens (e.g., *Salmonella* spp., anaerobic bacteria, *Campylobacter* spp., *Mycoplasma* spp.), recent antibiotic treatment, and culture for a secondary rather than a slow-growing primary pathogen (i.e., insufficient duration of culture). Failure to grow fastidious anaerobes may be caused by short, seemingly insignificant exposure to oxygen or failure to use prereduced culture media.

Interpretation • Recognizing normal flora (see Table 15-2) is necessary for correct interpretation. Preliminary identification is expected in 18 to 24 hours, and antibiotic sensitivity is reported in 36 to 48 hours. Most aerobic and facultative organisms are identified within 5 days; identification of anaerobic organisms or *Mycoplasma* spp. may require an additional 2 to 3 days.

Bacterial pathogens commonly isolated from various body systems are listed in Table 15-1. The overlap between resident and pathogenic organisms should be noted.

Staphylococcus intermedius is the major pathogen isolated from the skin of dogs with pyoderma. Gram-negative organisms are likely to be contaminants in superficial pyoderma and secondary to *S. intermedius* in deep pyoderma.

Primary bacterial rhinitis is rare in dogs and cats but can result from infection with *B. bronchiseptica*, *Mycoplasma* spp., and *Chlamydophila felis* (cats). Primary bacterial pneumonia can result from *Bordetella bronchiseptica* or *Mycoplasma* spp., whereas other organisms are usually secondary to viral infections or aspiration.

Bacterial growth from urine obtained by cystocentesis is significant because the bladder is normally sterile. Urine cultures, however, are best interpreted in conjunction with a urinalysis. If growth occurs despite absence of significant pyuria (see Chapter 7), sample contamination, improper sample transport, or diseases causing immune suppression (e.g., hyperadrenocorticism, diabetes mellitus, FIV infection) must be considered. In quantitative culture of urine obtained by catheterization or midstream voiding, greater than or equal to 100,000 colonies/ml is significant. Lower concentrations may be significant in chronic infections or in females. In samples of prostatic fluid obtained by ejaculation, infection is diagnosed if the specimen contains greater than or equal to 100 times more bacteria than the urethral sample (Ling et al, 1983). Culture of prostatic aspirates may be more accurate.

Blood cultures can be difficult to interpret. False-positive results are caused by contamination with normal cutaneous microflora, including *Corynebacterium* spp., *Bacillus* spp., coagulase-negative staphylococci, anaerobic diphtheroids, streptococci, and *Clostridium* spp. Isolation of the same organism from two or more cultures strongly suggests that it is pathogenic, whereas growth in only one culture is less certain unless it is a pathogenic bacterium unlikely to be a contaminant.

CSF and synovial fluid are normally sterile; any growth in an aseptically obtained sample is significant.

Fungal Culture

Specimen Procurement

For dermatophyte culture, hair is clipped from the lesion periphery; hair shafts are plucked with forceps and cultured on dermatophyte test medium (DTM)* or Derm Duet.†

SC and deep fungal infections are best diagnosed by cytologic or histopathologic evaluation, with or without serology. If organisms cannot be identified, cutaneous lesions can be cultured, but these are rarely useful owing to overgrowth by resident bacteria and fungi. The lesion is prepared as for dermatophytes, and a swab is cultured onto Sabouraud's and Mycose medium.

Systemic and SC fungi may require 2 weeks' cultivation on Sabouraud's medium for growth to occur.

SEROLOGIC TESTS FOR BACTERIAL INFECTIONS

Bartonellosis, Feline *(Bartonella henselae)*

Occasional Indications • Most cats with bartonellosis (*Bartonella henselae*) are subclinically infected; however, fever, uveitis, lymphadenopathy, gingivitis, or stomatitis occur in some cats and are indications for antibody testing. Because the organism is transmitted between cats by *Ctenocephalidies felis*, cats with a history of flea infestation are more likely to be infected. The clinical significance of other *Bartonella* spp. that infect cats is unknown at this time.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected by immunofluorescent antibody assay (IFA), enzyme-linked immunosorbent assay (ELISA), and western blot immunoassay. See Appendix I for availability of testing.

Cats infected by fleas are generally bacteremic by week 2; bacteremia can persist for months (Chomel et al, 1996). Antibodies are first detected at approximately week 2, and detectable concentrations persist for months after resolution of bacteremia. Bacteremia has also been detected in antibody-negative cats. *B. henselae* seroprevalence is often greater than 50% in regions with fleas; in healthy cats in a Birmingham, Alabama shelter, the seroprevalence was 71.4% (Lappin et al, 2000). Because so many healthy cats are seropositive, detection of positive antibody test results does not definitively diagnose clinical bartonellosis in a clinically ill cat; the clinician must still exclude other causes of the clinical syndrome. Because antibody test results do not correlate to blood culture results and because treatment of healthy carriers is not recommended (Lappin et al, 2003), screening healthy cats for *Bartonella* antibodies is not indicated. Detection of local antibody production by the eye has been used to document uveitis as a result of bartonellosis (Lappin et al, 2000).

Definitive diagnosis is based on detection of the organism in blood. Culture for *B. henselae* is generally performed on a 1.5 ml whole blood sample collected aseptically and placed into an EDTA-containing tube. Organism DNA can be amplified from blood or aqueous humor by PCR assay (Jensen et al, 2000; Lappin et al, 2000). Positive blood culture or PCR results are consistent with current infection but do not document clinical illness. Repeated bacteremia has been detected in experimentally inoculated and naturally infected cats (Kordick et al, 1995); therefore, a single negative blood culture or PCR result does not exclude infection. See Appendix I for availability of *Bartonella* spp. blood culture and PCR.

Bartonellosis, Canine *(Bartonella vinsonii)*

Occasional Indications • Dogs from endemic areas or with an appropriate travel history with unexplained myocarditis, granulomatous lymphadenitis, cutaneous vascular disease, hemolytic anemia, polyarthritis, granulomatous meningoencephalitis, or thrombocytopenia should be considered for *B. vinsonii* serologic screening. Based on seroprevalence studies, rural dogs with fleas or ticks are most likely to be exposed.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected by IFA. See Appendix I for availability of testing.

Antibodies can be detected in dogs with and without clinical signs. Seronegative test results make clinical illness caused by *B. vinsonii* less likely. Seropositive test results suggest that

*Dermatophyte test media, Pittman Moore, Mundelein, Ill.
†Derm Duet, Bacti Lab., Mountain View, Calif.

the organism could be involved, but the clinician must still exclude other causes of the clinical syndrome.

Definitive diagnosis is based on detection of the organism in blood, but the organism is more difficult to culture than *B. henselae*. Amplification of the organism DNA is usually more successful than culture. (See Appendix I for availability of testing.) Positive blood culture or PCR results are consistent with current infection but do not document clinical illness. A single negative blood culture or PCR does not exclude infection by *B. vinsonii*.

Borreliosis (Lyme Disease) *(Borrelia burgdorferi)*

Occasional Indications • Dogs from areas endemic for *Ixodes* ticks or with an appropriate travel history and fever, lameness, glomerulonephritis (Dambach et al, 1997) or nonseptic, suppurative polyarthritis should be suspected of having Lyme disease (borreliosis) and screened for antibodies against *Borrelia burgdorferi*. Serologic testing should be considered in dogs with CNS disease, renal disease, and myocardial disease.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA, ELISA, and Western immunoblot. See Appendix I for availability of testing.

IgM and IgG antibodies against *B. burgdorferi* can be detected in canine serum. Titers considered significant vary by laboratory and assay. Both antibody classes can persist in serum for months after exposure. Cross-reactivity with *B. burgdorferi* antigens used in IFA and ELISA occurs with other spirochetes; thus, a positive titer does not document exposure to *B. burgdorferi*. *B. burgdorferi* vaccines induce antibodies that are detected by IFA and ELISA and one of the available point-of-care assays.* Western immunoblot can differentiate vaccine-induced antibodies from those from natural infection (Jacobsen, Chang, and Shin, 1996). A point-of-care kit† commercially available for detection of *B. burgdorferi* antibodies against the C6 peptide, *E. canis* antibodies, and *Dirofilaria immitis* antigen was licensed recently. Antibodies against the C6 peptide are not induced by vaccination, so positive results of this kit denote exposure to *B. burgdorferi*. Some nonpathogenic strains of *B. burgdorferi* induce antibody production but not clinical disease (Breitschwerdt, 1995). Some dogs with acute Lyme disease are seronegative on initial testing; documentation of an increasing antibody titer can suggest recent exposure. Antibody titers greater than or equal to 1:1000 have been detected in clinically normal dogs. Healthy dogs develop the same antibody responses as clinically ill dogs, however. Because of these factors, interpretation of serum antibody titers is difficult. Serum antibodies against *B. burgdorferi* only documents exposure to *B. burgdorferi* (or a similar antigen), not clinical disease. Finding a higher titer in CSF than in serum occurs in some dogs with suspected neurologic disease secondary to Lyme disease.

Definitive diagnosis requires demonstration of the organism by culture, histopathologic evaluation of tissue, or PCR. Presumptive diagnoses of clinical Lyme disease in dogs can be based on appropriate clinical, historical, and laboratory evidence of disease combined with positive serologic testing and response to therapy.

Brucellosis *(Brucella canis)*

Occasional Indications • Dogs with reproductive tract abnormalities, lymphadenomegaly, hyperglobulinemia, diskospondylitis, or uveitis should be suspected of having brucellosis and screened for antibodies against *B. canis*.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by rapid slide agglutination test (RSAT), tube agglutination test (TAT), agar gel immunodiffusion (AGID), and ELISA (Carmichael and Shin, 1996).

The RSAT and TAT are screening procedures; a RSAT for point-of-care use is commercially available.* Both assays should be performed with 2-mercaptoethanol (2-ME) to eliminate heterologous IgM agglutinins responsible for most false-positive reactions. False-positive reactions in the 2-ME TAT may be the result of autoagglutination in hemolyzed samples. AGID can be performed using cell wall antigens or cytoplasmic antigens. AGID performed with cytoplasmic antigens is the most specific

*LymeCHEK *Borrelia burgdorferi* antibody test, Synbiotics Corp, San Diego, Calif.
†Snap3Dx, IDEXX Laboratories, Portland, Me.

*D-Tec CB, Synbiotics Corp, San Diego, Calif.

antibody assay; AGID performed with cell wall antigens is the most sensitive. Because of nonspecific precipitin reactions, positive results in AGID with cell wall antigens are difficult to interpret.

Minimal time between infection and a positive test result varies with the test, but most infected dogs are seropositive in the 2-ME TAT and AGID by week 4 after infection. 2-ME TAT titers from different laboratories cannot be meaningfully compared; however, a titer of 1:50 to 1:100 is generally suspicious, whereas a titer greater than or equal to 1:200 usually correlates with isolation of *B. canis* from blood culture (Carmichael and Shin, 1996). After cessation of bacteremia, 2-ME TAT titers rapidly decrease to less than 1:200 within a few weeks and remain low (1:25 to 1:50) for 6 months or longer. In AGID, antibodies to external antigens persist for a few weeks, whereas antibodies to internal (i.e., cytoplasmic) antigens persist up to 12 months after cessation of bacteremia. Although these animals are abacteremic, *B. canis* can be isolated from selected organs (e.g., epididymis, prostate).

When the 2-ME RSAT or TAT are used as a screening test and results are positive, a tentative diagnosis of brucellosis is made; positive blood culture or AGID should be used to confirm results. If blood culture or AGID is negative, brucellosis is unlikely. If 2-ME RSAT or TAT results are negative in a dog strongly suspected of having brucellosis, the test should be repeated in 4 weeks to preclude the possibility of early infection.

Definitive diagnosis requires isolation of *B. canis*, although this is not always achieved. Although blood culture is ideal, it is inconvenient and expensive. Culture of urine or an ejaculate may also be performed in males. Growth usually occurs within 7 days, but cultures should be held for 3 to 4 weeks before being discarded. At least three cultures from specimens obtained several days apart are recommended.

Leptospirosis (*Leptospira* spp.)

Occasional Indications • Serologic testing for antibodies against *Leptospira* spp. should be considered in dogs with undiagnosed fever, ecchymoses, vomiting, diarrhea, muscle pain, uveitis, coughing, dyspnea, renal pain, thrombocytopenia, renal failure (particularly acute), or increased activities of hepatic enzymes. The most common pathogenic serovars in dogs include *Leptospira canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. bratislava,* and *L. pomona* (Rentko and Ross, 1992).

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by the microscopic agglutination test (MAT), ELISA (IgM, IgG), and microscopic microcapsular agglutination test (MCAT). Most diagnostic laboratories use MAT (Bolin, 1996). The primary disadvantage of serologic testing is that it is difficult to determine whether positive titers are caused by active infection, previous infection, or vaccination.

Antibodies are detected by MAT days to weeks after inoculation. Most laboratories assess multiple serovars in the assay. Cross-reactivity exists between some serovars; the serovar with the highest titer is probably the serovar causing infection. Acutely infected dogs are often MAT negative; dogs with suggestive clinical signs of disease but negative MAT results should be retested in 2 to 4 weeks; development of a positive titer confirms recent infection. A fourfold increase in antibody titer over 2 to 4 weeks confirms recent infection. Vaccination can induce positive MAT titers.

Definitive diagnosis requires demonstration of the organism by urine dark-field microscopy, phase contrast microscopy, or culture. Examination of urine for leptospires is a low-yield procedure. Demonstration of spirochetes by histopathologic evaluation of renal tissue leads to a presumptive diagnosis, which may be confirmed by tissue culture. In acute disease, leptospiremia occurs for a few days to 2 weeks after infection, during which time the organism may sometimes be cultured from blood. Urine can be cultured, but repeated culture may be needed because of intermittent shedding. The combination of increasing antibody titers with appropriate clinical pathologic abnormalities and clinical findings suggests clinical leptospirosis. PCR can be used to demonstrate the organism in urine, blood, or tissues, but is not widely available at this time, is not standardized between laboratories, and is expensive.

Tularemia (Rabbit Fever) (*Francisella tularensis*)

Rare Indications • Testing for tularemia (i.e., Rabbit fever) should be considered in animals from endemic areas developing fever, lymphadenomegaly, weight loss, or

oral ulceration, particularly if tick exposure, rabbit ingestion, or potential for human infection is confirmed. Tularemia is a direct zoonosis from clinically ill cats to people.

Analysis, Artifacts, and Interpretation • Clinicians measure antibodies in serum using TAT. Cross-reactivity with *Brucella abortus* and certain strains of *Proteus vulgaris* has been documented with human serum. (See Appendix I for availability of testing.) Time between acquisition of infection and a positive titer is not known. A single titer of 1:80 or higher or a fourfold increase in titer between acute and convalescent sera (3 weeks later) is presumptive evidence of infection. Definitive diagnosis is obtained by isolation of the bacterium in a culture of a blood specimen or by identification in tissue by immunofluorescence.

SEROLOGIC TESTS FOR FUNGAL INFECTIONS

Aspergillosis *(Aspergillus fumigatus)*

Occasional Indications • Dogs and cats with nasal or pulmonary disease can be serologically screened for antibodies against *Aspergillus fumigatus*; cats are affected less frequently than dogs. Results must be interpreted in conjunction with cytology, radiology, histopathology, and culture.

Analysis, Artifacts, and Interpretation • AGID, counterimmunoelectrophoresis (CIEP), and ELISA are used to detect circulating antibodies in serum (Sharp, 1998). Serum antibodies can represent exposure or infection. Results in many dogs with nasal aspergillosis are falsely negative. Owing to persistence of titers in some treated dogs (12 months), monitoring titers to assess therapeutic response is not recommended.

Radiographic demonstration of nasal turbinate destruction suggests aspergillosis or nasal neoplasia. Cytologic analysis (see Color Plate 3D) and culture of canine nasal exudate alone are not diagnostic because fungal elements may be nondetectable in affected dogs although being found in noninfected dogs (including dogs with nasal tumors). The organism is sometimes difficult to culture from an aspergilloma (fungal ball). Nasal lavage is a low-yield procedure for demonstration of the organism. Nasal biopsy is suggested (see Chapter 11). Definitive diagnosis should be based on three factors: (1) histopathologic evidence of tissue invasion, (2) an aspergilloma combined with serologic and culture evidence of infection, or (3) serologic and radiographic evidence of infection (i.e., bone lysis). In rare cases with disseminated disease, cytologic evaluation of aspirates of affected tissue may be useful. If the organism cannot be demonstrated by biopsy samples obtained through the nares, positive serologic test results may support exploratory surgery.

Blastomycosis *(Blastomyces dermatitidis)*

Occasional Indications • Dogs from endemic areas with fever, weight loss, pulmonary interstitial disease, lymphadenomegaly, uveitis and blindness, ulcerative or draining skin lesions, undiagnosed prostatic or testicular disease, intracranial disease, osteomyelitis, or (rarely) renal disease can be serologically screened for antibodies against *Blastomyces dermatitidis* if the organism is not demonstrated by cytology, histopathology, or culture. In endemic areas, screening for antibodies against *B. dermatitidis* should be considered in cats with pulmonary interstitial disease, intracranial disease, lymphadenomegaly, ulcerative or draining skin lesions, or uveitis and blindness.

Analysis, Artifacts, and Interpretation • Circulating antibodies are most commonly detected in serum by AGID (Legendre, 1998). Because subclinical canine infections are unusual, positive serologic results are considered significant. False-negative results occur in animals with peracute infection or with advanced cases overwhelming the immune system. Many cats with blastomycosis are seronegative. Antibody titers do not always revert to negative after successful treatment.

Definitive diagnosis requires identification of the yeast by cytology, histopathology, or fungal culture. Impression smears from skin lesions and aspirates from enlarged lymph nodes frequently reveal organisms; recovery of organisms from transtracheal aspiration, pulmonary aspiration biopsy samples, or urine is less consistent. Culture requires 10 to 14 days and is of lower yield than cytology or biopsy. Diffuse nodular interstitial pulmonary disease and hilar lymphadenomegaly are common radiographic findings. Positive serologic results combined with appropriate clinical

signs and radiographic abnormalities allow presumptive diagnosis.

Coccidioidomycosis *(Coccidioides immitis)*

Occasional Indications • Dogs from endemic areas with pulmonary interstitial disease, fever of undetermined origin, hilar lymphadenopathy, osteomyelitis, uveitis, pericarditis, and nodular or ulcerative skin lesions can be screened for antibodies against *Coccidioides immitis* if the organism is not demonstrated by cytology, histopathology, or culture. Feline disease is rare but has been associated with nodular or ulcerative skin lesions, pulmonary interstitial disease, osteomyelitis, uveitis, and CNS disease.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by complement fixation (CF), AGID, ELISA, latex agglutination, and tube precipitin (TP) tests. TP detects IgM antibodies; CF and AGID detect IgG antibodies (Greene, 1998). False-negative results in TP occur in early infections (< 2 weeks), chronic infection, rapidly progressive acute infection, and primary cutaneous coccidioidomycosis. False-positive results in the CF test are caused by anticomplementary serum, which may be caused by bacterial contaminants or immune complexes. Finally, cross-reactions in patients with histoplasmosis and blastomycosis may occur with all tests. After resolution of disease, CF titers decrease over weeks but remain positive at a low titer (e.g., 1:32) for months.

Definitive diagnosis requires demonstration of the organism on smears, aspirates, histopathologic evaluation, or culture. The organism is often difficult to demonstrate. Wet-mount examination of unstained or stained (periodic acid-Schiff) smears or aspirates is more suitable than dry mounts, which may distort the spherules. Common thoracic radiographic findings are mixed interstitial, bronchial, and alveolar pulmonary patterns and hilar lymphadenomegaly. Positive serologic test results and characteristic radiographic changes allow tentative diagnosis.

Cryptococcosis *(Cryptococcus neoformans)*

Occasional Indications • Cats and rarely dogs with undiagnosed respiratory (especially nasal), CNS, eye (especially uveal tract), and skin (especially nodular or ulcerative lesions) infections can be screened for *C. neoformans* antigen if the organism is not demonstrated by cytology, histopathology, or culture.

Analysis, Artifacts, and Interpretation • Measurement of antibodies against *C. neoformans* is not clinically useful. Cryptococcal antigen is detected in serum, aqueous humor, or CSF using latex agglutination (LA).*

Negative serum LA titers may occur in early disease or uncommonly in chronic low-grade infections, in chemotherapy-induced remission, or in nondisseminated disease. Specificity of the serum LA is high. A titer of greater than 1:1 in serum or CSF is positive; very high titers are commonly detected. In some animals, decreases in serum titer parallel response to therapy (Malik et al, 1996a). Positive titers occur in some animals after apparently successful clinical responses suggesting persistent low-grade infection or false-positive results (Flatland, Greene, and Lappin, 1996; Jacobs et al, 1997). Cryptococcal encephalitis may cause a positive CSF LA titer despite a negative serum LA.

Definitive diagnosis is based on cytologic, histopathologic, or culture demonstration of the organism or a positive LA test result. Cytology is commonly positive (Color Plate 4E) because there are usually numerous yeasts found in affected tissues (i.e., nasal and cutaneous lesions, aqueous and vitreous humor). **NOTE:** The organism can occasionally be recovered from nasal washings of normal animals (Malik et al, 1996b). CSF may contain the yeast, but concentration techniques (i.e., cytocentrifugation) should be used. Routine cytology stains (e.g., Wright's) are adequate to demonstrate the organism. Large numbers of organisms are usually visible despite little or no inflammation. Culture is seldom necessary. Serologic testing is used if the yeast cannot be demonstrated cytologically or to monitor response to treatment. A PCR assay has been used to amplify the organism DNA from tissue but has not been assessed extensively to date (Kano et al, 2001).

Histoplasmosis *(Histoplasma capsulatum)*

Rare Indications • Animals with weight loss, pulmonary interstitial disease, uveal disease, diarrhea, or lymphadenomegaly can be serologically screened for antibodies against *H. capsulatum* if the organism is not

*Cryptococcus latex agglutination test, Cima Scientific, Dallas, Tex.

demonstrated by cytology, histopathology, or culture (Wolf, 1998).

Analysis, Artifacts, and Interpretation • Primarily, AGID is used to detect circulating antibodies in serum. Presence of serum antibodies confirm exposure but not clinical illness because of infection. AGID has questionable clinical usefulness because titers persist longer than 1 year after resolution of disease in some animals, and both false-positive and false-negative results occur. Antibody testing is even less rewarding in cats.

Definitive diagnosis requires demonstration of the organism by cytology (see Color Plate 3E), biopsy, or culture. The organism is more difficult to demonstrate than *B. dermatitidis*; however, cytologic examination of rectal scrapings in dogs with colonic histoplasmosis is often diagnostic. Fine-needle aspiration of other organs may demonstrate the organism. In most cats with systemic histoplasmosis, the organism is identified on bone marrow cytology. Thoracic radiographs are indicated if pulmonary histoplasmosis is suspected; a nodular interstitial pattern is expected. Culture of *H. capsulatum* is of lower yield than biopsy. Serologic diagnosis is unreliable, generally not recommended, and used only to establish a presumptive diagnosis when the organism cannot be demonstrated by cytology, histopathology, or culture but other abnormalities suggest the disease.

SEROLOGIC TESTS FOR PROTOZOAL INFECTIONS

Babesiosis (*Babesia canis canis, B. canis rossi, B. canis vogeli,* and *B. gibsoni* in Dogs; *B. cati, B. felis, B. herpailuri,* and *B. pantherae* in Cats)

Rare Indications • Babesia serology is indicated in dogs from endemic areas or in those with an appropriate travel history that have fever, anemia, icterus, splenomegaly (i.e., acute babesiosis), or intermittent fever and weight loss (i.e., chronic babesiosis). Although babesiosis can cause anemia in cats, the species infecting cats are not found in the United States.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA. (See Appendix I for availability of testing.) In most laboratories, titers greater than 1:40 are considered positive (Breitschwerdt, 1995). Experimentally infected dogs develop detectable IgG titers approximately 3 weeks after infection. False-negative results can occur in immature dogs, in peracute cases, or in dogs with concurrent immunosuppression. Antibodies against *B. gibsoni* and *B. canis* may or may not cross-react, depending on the antigen source used by a particular laboratory. Some dogs with *B. gibsoni* are seronegative on IFA using *B. canis* antigen; IFAs are available using *B. gibsoni* antigens. Dogs suspected of having babesiosis yet seronegative for *B. canis* should be screened for antibodies against *B. gibsoni*. Antibodies can be detected in dogs that are healthy and those that are clinically ill. Antibody titer magnitude does not correlate to the presence or absence of disease. A titer of greater than 1:320 was suggested for *B gibsoni*, but not all infected dogs achieve this titer magnitude (Birkenheuer et al, 1999). It is important to determine which species are involved in a case because response to treatment varies. Duration of positive titers after resolution of disease is unknown. In untreated experimentally infected dogs, titers remain high for at least 6 months. Untreated, seropositive dogs should be considered carriers of the infection. Treatment is indicated only for seropositive, clinically ill dogs.

Definitive diagnosis requires demonstration of the organism in blood smears stained with Romanowsky-type preparations (e.g., Wright's and Giemsa's). Organisms are best found in blood (particularly in acute disease) from a microcapillary system (e.g., ventral surface of ear or toenail). **NOTE:** Shape of the organism may be distorted in old blood. In chronic disease or asymptomatic carriers, demonstration of organisms is unreliable, and a tentative diagnosis is based on clinical signs and a positive titer. Dogs with babesiosis are often Coombs'-positive (see Chapter 3). PCR is now available commercially and can be used to document organism presence, but positive results do not always correlate to clinical illness (Ano, Makimura, and Harasawa, 2001).

Neosporosis (*Neospora caninum*)

Rare Indications • Serology for *N. caninum* (the cause of neosporosis) can be performed in dogs with clinical evidence of polyradiculomyositis, including progressive ascending rigid paralysis, dysphagia, muscle atrophy, and (rarely) myocardial dysfunction or pneumonia (Lindsay and Dubey, 2000).

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA. (See Appendix I for availability of testing.) A presumptive diagnosis of neosporosis can be made by combining appropriate clinical signs of disease and positive serology or presence of antibodies in CSF with the exclusion of other causes inducing similar clinical syndromes, in particular, *T. gondii*. IgG antibody titers greater than or equal to 1:200 have been detected in most dogs with clinical neosporosis; minimal serologic cross-reactivity exists with *T. gondii* at titers greater than or equal to 1:50. Because the organism is a tissue protozoan, seropositivity may correlate with permanent infection. Circulating antibodies against *N. caninum* only documents infection, not clinical disease.

Definitive diagnosis is based on demonstration of the organism in tissues. The organism can be differentiated from *T. gondii* structurally and by immunohistochemistry (Lindsay and Dubey, 1999). *Neospora caninum* DNA can be amplified from tissue by PCR assay, and PCR can be used to distinguish the organism from *T. gondii*. *Neospora caninum* oocysts are found in the feces of some dogs (McAllister et al, 1998).

Toxoplasmosis *(Toxoplasma gondii)*

Occasional Indications

Healthy cats: Toxoplasma gondii–specific antibodies form in serum, aqueous humor, and CSF of healthy and diseased cats. Antibodies do not directly correlate with clinical toxoplasmosis. No serologic test is currently available that accurately predicts when a seropositive cat previously shed oocysts. A seropositive cat is less likely than a seronegative cat to shed the organism if re-exposed.

Clinically ill dogs and cats: Serologic tests for toxoplasmosis should be considered in cats with uveitis, fever, muscle disease, icterus, pancreatitis, apparent inflammatory bowel disease failing to respond to immunosuppressive therapy, CNS disease, and respiratory disease. Serologic tests for toxoplasmosis should be considered in dogs with fever, muscle disease, CNS disease, and respiratory disease. Dogs develop clinical toxoplasmosis less commonly than cats.

Analysis

Serum Antibody Testing • Antibodies against *T. gondii* can be detected with multiple techniques including ELISA, IFA, Western blot immunoassay, Sabin-Feldman dye test, and various agglutination tests (Lappin, 1996). See Appendix I for availability of testing.

ELISA, IFA, and Western blot immunoassay can be adapted to detect various antibody classes; IgM and IgG are those usually assessed. *T. gondii*–specific IgM is detectable in serum by ELISA in approximately 80% of subclinically ill cats 2 to 4 weeks after experimental induction of toxoplasmosis; these titers generally are negative less than 16 weeks after infection. Detectable IgM titers were present in the serum of 93.3% of cats in a study of clinical toxoplasmosis; IgG titers were detected in 60% (Lappin, 1996). IgM titers persist in some clinically ill cats for greater than 16 weeks; these cats are frequently coinfected with FIV or have ocular toxoplasmosis. After repeat inoculation with *T. gondii*, primary inoculation with the Petaluma isolate of FIV, and administration of glucocorticoids, some cats with chronic toxoplasmosis experience short-term recurrence of detectable IgM titers (Lappin, 1996). Healthy and clinically ill dogs occasionally develop detectable IgM titers. Kinetics of post-infection IgM titers in dogs is unknown.

After experimental induction of infection in subclinically ill cats, *T. gondii*–specific IgG can be detected by ELISA in serum from most cats by 4 weeks. Positive IgG antibody titers generally persist for years after infection. Single high IgG titers have been suggested to indicate recent or active infection. The authors, however, have demonstrated IgG antibody titers greater than 1:16,384 in subclinically ill cats 5 years after experimental induction of toxoplasmosis. A positive IgG antibody titer in a single serum sample only documents exposure, not recent or active disease. Demonstration of an increasing IgG titer can document recent or active disease. Unfortunately, the time span from the first detectable positive IgG titer to the maximal IgG titer is approximately 2 to 3 weeks, leaving a very narrow window for documenting an increasing titer. Many cats with clinical toxoplasmosis have chronic low-grade signs, and they are not tested until their IgG antibody titers have reached maximal values. In humans with reactivation of chronic toxoplasmosis, IgG titers only rarely increase; cats appear to be the same.

Several agglutination tests have been evaluated using cat serum. A LA* and an indirect

*Toxotest-MT, Tanabe USA, Inc, San Diego, Calif.

hemagglutination assay (IHA)[†] are commercially available. These assays are not species specific and potentially detect all classes of serum immunoglobulins directed against *T. gondii*. Unfortunately, LA and IHA rarely detect antibody in feline sera when positive for only IgM by ELISA. Modified agglutination using formalin-fixed tachyzoites is the most sensitive procedure for detection of *T. gondii* antibodies in cat sera, but it is generally unavailable commercially.

Aqueous Humor and CSF Antibody Measurement • Local production of *T. gondii*-specific IgG in CSF and aqueous humor occurs in experimentally inoculated, subclinically ill cats and in cats and dogs with clinical disease because of toxoplasmosis.

Local IgM production has only been detected in CSF and aqueous humor of animals with clinical disease. Most cats with uveitis and production of *T. gondii*-specific antibodies in aqueous humor have responded to administration of anti-*Toxoplasma* drugs, suggesting that aqueous humor antibody testing aids in diagnosis of clinical ocular feline toxoplasmosis. See Appendix I for availability of testing.

Fecal Examination • Fecal oocysts can be demonstrated using flotation techniques with various solutions with specific gravities 1.15 to 1.18. Sugar solution centrifugation is probably the optimal technique. Oocysts of *T. gondii* are 10 to 12 μm in diameter, approximately one eighth the size of *Toxocara cati* eggs. Focusing on only one plane of the microscope slide or coverslip can result in oocysts being overlooked. The oocysts cannot be distinguished grossly from *Hammondia hammondi* or *Besnoitia darlingi* (nonpathogenic coccidians infecting cats). Sporulated oocysts isolated from feces can be inoculated into mice or tissue cultures for definitive identification. Because oocyst shedding has rarely been documented in cats with subfatal, clinical toxoplasmosis, the diagnostic usefulness of fecal examination is limited. Cats with clinical signs referable to *T. gondii* should undergo fecal evaluation, however, because of potential zoonotic risk.

Interpretation • Exposure to *T. gondii* is suggested by finding antibodies in serum, aqueous humor, or CSF. Recent or active toxoplasmosis is suggested by finding an IgM titer greater than 1:64, a fourfold or greater increase in IgG titer, or documenting local antibody production in aqueous humor or CSF. Because *T. gondii*-specific antibodies can also be detected in the serum, CSF, and aqueous humor of healthy, infected animals, one cannot base an antemortem diagnosis of clinical toxoplasmosis on these tests alone. Antemortem diagnosis of clinical toxoplasmosis can be tentatively based on the combination of the following:

- Demonstration of serologic evidence of infection
- Clinical signs of disease referable to toxoplasmosis
- Exclusion of other common causes
- Positive response to appropriate treatment

T. gondii was detected by PCR in aqueous humor of 18.6% of cats with uveitis (Lappin, 1996). The organism also can be detected transiently in aqueous humor and blood of healthy, experimentally inoculated cats (Lappin, 1996); however, making the positive predictive value of the PCR for clinical disease less than 100%.

Trypanosomiasis (Chagas' Disease) *(Trypanosoma cruzi)*

Rare Indications • Serologic testing for antibodies against *T. cruzi* should be considered in dogs from endemic areas and those with generalized lymphadenomegaly, neurologic signs, or myocardial dysfunction (especially second- or third-degree heart block or ventricular tachycardia).

Analysis, Artifacts, and Interpretation • IFA, direct hemagglutination, and CF usually detect circulating antibodies in canine sera (Barr, 1998). See Appendix I for availability of testing.

Dogs are generally seropositive 3 weeks after infection. A positive titer documents exposure to the organism, not clinical disease. Positive titers vary by assay. Definitive diagnosis requires demonstration of the organism on blood smear, lymph node impression, or buffy coat and plasma interface smear. *T. cruzi* is occasionally found in peripheral blood without demonstrable organisms in tissue. A standard workup for myocardial disease, including chest radiographs, electrocardiogram, electrolytes, and echocardiography (if available), is indicated. Alternatively, *T. cruzi* amastigotes

[†]TPM-Test, Wampole Laboratories, Cranbury, N.J.

can be demonstrated in tissues. PCR can be used to amplify organism DNA.

SEROLOGIC TESTS FOR RICKETTSIAL INFECTIONS

Canine Ehrlichiosis *(Ehrlichia canis)*

Common Indications • Serologic testing for ehrlichiosis is indicated for dogs from endemic areas or with an appropriate travel history and thrombocytopenia, anemia, leukopenia, hyperglobulinemia, proteinuria, polyarthritis, fever, uveitis, lymphadenomegaly, hepatosplenomegaly, or inflammatory CNS disease, particularly if the animal has a history of exposure to *Rhipicephalus* ticks.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA; they do not cross-react with *Rickettsia rickettsii* or *E. platys* antigens. *Anaplasma phagocytophila* (previously *Ehrlichia equi*), *E. chaffeensis, E. risticii*, and *E. ewingii* (Anderson et al, 1992) can also cause disease in dogs. Cross-reactivity of antibodies against these agents with *E. canis* antigen varies, and infected dogs can be serologically negative. See Appendix I for availability of testing for antibodies against other Ehrlichial species.

Antibodies against *E. canis* can be detected as early as 7 days and are almost always present by 28 days after inoculation (Neer et al, 2002). Antibody titers continue to increase for weeks to months after inoculation in untreated, experimentally infected dogs. *E. canis* titers of less than 1:80 are suspect and should be rechecked in approximately 14 to 21 days; a titer of 1:80 or higher is diagnostic. Initial positive results in a recently marketed point-of-care test* occur at approximately 1:100. Positive titers revert to negative 3 to 9 months after resolution of infection; persistence of titers for greater than or equal to 9 months suggests a carrier state. However, positive antibody titers have been detected for months after apparently successful therapy in some naturally infected dogs (Bartsch and Greene, 1996). Clinically ill, seropositive dogs should be treated a minimum of 28 days and until clinical and laboratory abnormalities have resolved (Neer et al, 2002). Whether to treat healthy, seropositive dogs is controversial; the issues involved in this decision were recently reviewed (Neer et al, 2002).

*Snap3Dx, IDEXX Laboratories, Portland, Me.

The clinician can make a definitive diagnosis of *E. canis* infection by demonstrating morulae (i.e., clusters of the organism) in mononuclear cells, culture, or PCR. Morulae are rarely found on routine blood smear or bone marrow aspiration cytology unless the dog has been immunosuppressed or the neutrophilic strain *(E. ewingii)* is present. *Ehrlichia* spp. can be isolated by tissue culture of heparinized infected canine blood or bone marrow aspiration samples, but culture is of limited availability, expensive, and of low yield. *Ehrlichia* spp. can be detected in whole blood by PCR (McBride et al, 1996) and has potential benefit for use in monitoring treatment. (See Appendix I for availability of testing.) The *Consensus Statement on Ehrlichial Disease of Small Animals* from the Infectious Disease Study Group of the ACVIM (Neer et al, 2002) states the following:

If PCR is used to monitor treatment, the PCR assay should be repeated after antimicrobial therapy has been discontinued for 2 weeks. If PCR results are positive, an additional 4 weeks of treatment should be given with the PCR assay repeated after antimicrobial therapy has been discontinued for 2 weeks. If PCR results are positive after 2 treatment cycles, use of an alternate antiehrlichial drug should be considered. If PCR results are negative the test should be rechecked in 2 months; if still negative therapeutic elimination is likely. However, the organism may be sequestered in other tissues like the spleen.

Feline Ehrlichiosis (*Ehrlichia* spp.)

Rare Indications • Serologic testing for ehrlichiosis is indicated for cats with thrombocytopenia, anemia, leukopenia, hyperglobulinemia, proteinuria, polyarthritis, fever, or lymphadenomegaly if no other obvious cause exists (Bouloy et al, 1994; Peavy et al, 1997; Stubbs et al, 2000).

Analysis, Artifacts, and Interpretation • IFA has detected circulating IgG antibodies against *E. canis*, *E. risticii*, and *Anaplasma phagocytophila* (previously *E. equi*) in serum of cats. PCR has detected DNA of *E. canis* (Breitschwerdt et al, 2003) and *A. phagocytophila* (Lappin et al, 2003) in the blood of clinically ill cats. Some cats with presumed *E. canis* infection have been seronegative. Antibodies against *E. canis, E. risticii*, and *A. phagocytophila* can be detected in serum from healthy cats

and therefore cannot be used alone to make a definitive diagnosis of ehrlichiosis. A tentative diagnosis of feline ehrlichiosis is based on the combination of clinical signs, positive serologic test results, exclusion of other known causes, and response to tetracyclines. Definitive diagnosis is based on demonstration of morulae in leukocytes or amplification of DNA from the blood by PCR. See Appendix I for availability of PCR testing.

Infectious Cyclic Thrombocytopenia *(Ehrlichia platys)*

Occasional Indications • Serologic testing for *E. platys* infection is indicated for dogs from endemic areas or with appropriate travel history and thrombocytopenia or endogenous uveitis.

Analysis, Artifacts, and Interpretation • Circulating IgG antibodies against *E. platys* are detected in serum by IFA. Antibodies against *E. platys* do not react with *E. canis* antigens. (See Appendix I for availability of testing.) The cutoff for a positive IgG antibody titer varies by laboratory. Experimentally infected dogs become antibody positive 13 to 19 days after infection (French and Harvey, 1983). Low antibody titers in suspected clinical cases should be rechecked after 21 days. Because many dogs are subclinically infected, positive antibody titers do not prove clinical disease. Definitive diagnosis requires demonstration of the organism within platelets (difficult, owing to cyclic parasitemia).

Rocky Mountain Spotted Fever *(Rickettsia rickettsii)*

Occasional Indications • Serologic testing for RMSF is indicated for dogs from endemic areas or with an appropriate travel history and acute onset of fever, lymphadenomegaly, petechiae, neurologic signs, stiff gait, peripheral edema, dyspnea, or scleral congestion. History of tick exposure is inconsistent. Exposed dogs either develop acute disease with approximately a 14-day clinical course or are subclinically infected. The primary tick vectors are active from spring to fall in most of the United States; therefore, RMSF should only be considered a principal differential diagnosis for clinically ill dogs during this time span. The majority of cases are diagnosed in Southeastern states.

Analysis, Artifacts, and Interpretation • The clinician can measure antibodies against *R. rickettsii* in canine serum by IFA, ELISA, and LA (Greene et al, 1993). ELISA or IFA can detect IgM and IgG antibodies against RMSF. LA is not antibody class-specific. Cutoffs for positive antibody titers, as well as specificity and sensitivity, vary by assay (Greene et al, 1993). After experimental inoculation, IgM antibodies can be detected by IFA by day 9, peak by day 20, and are negative by day 80 (Breitschwerdt, 1995). In dogs with clinical illness because of RMSF, IgM antibody titers are generally positive. Because IgM has short duration in serum, false-negative results may occur with IgM testing. False-positive results are most common in the IgM ELISA. Positive IgG titers are detectable 20 to 25 days after infection. Serum samples with IgG titers greater than or equal to 1:64 are generally considered positive. If IgG or IgM antibodies are not detected in a patient with clinical and laboratory evidence of RMSF, a convalescent IgG titer 2 to 3 weeks later is recommended. Timing of the second titer is not critical because IgG antibody titers do not decrease for at least 3 to 5 months after infection. Documentation of seroconversion or a four-fold increase in IgG titer is consistent with recent infection.

A presumptive diagnosis of canine RMSF can be based on the combination of appropriate clinical, historical, and clinicopathologic evidence of disease; serologic test results; exclusion of other causes of the clinical abnormalities; and response to anti-rickettsial drugs. Documentation of seroconversion or an increasing titer 2 to 3 weeks after initial serologic testing suggests recent infection. Diagnostic criteria used in one recent study included a four-fold rise in antibody titer or a single titer of greater than or equal to 1:1024 if the initial titer was submitted 1 week or more after initial onset of clinical abnormalities (Gasser, Birkenheuer, and Breitschwerdt, 2001). Positive serum antibody test results alone do not prove RMSF because subclinical infection is common. In addition, positive serum antibody tests do not document infection by *R. rickettsii* because infection with nonpathogenic spotted fever group agents induce cross-reacting antibodies.

Demonstration of *R. rickettsii* by inoculating affected tissues or blood into susceptible laboratory animals or by documenting the organism in endothelial cells using direct fluorescent antibody staining leads to a definitive

diagnosis of RMSF but are not clinically practical. PCR can be used to document the presence of rickettsial agents in blood, other fluids, or tissues and will likely be clinically useful in the future.

SEROLOGIC TESTS AND IDENTIFICATION TECHNIQUES FOR VIRAL INFECTIONS

Canine Distemper

Rare Indications • Dogs with appropriate signs of CNS disease can have antibodies in CSF and serum against canine distemper virus.

Analysis, Artifacts, and Interpretation • The clinician can measure CSF and serum IgG antibodies against canine distemper virus by serum virus neutralization, IFA, or ELISA. ELISA can be used to measure serum IgM antibodies. CSF antibodies to distemper virus are increased in some dogs subsequently diagnosed histopathologically as having distemper encephalitis. False-positive results can occur in CSF samples contaminated with blood. Concurrent measurement of serum antibody concentrations can be helpful; if CSF concentrations are greater than serum concentrations, the antibody in CSF had to be produced locally and suggests CNS distemper. Detection of serum IgG antibodies is of minimal diagnostic value because a positive titer could develop secondary to vaccination or previous exposure. A fourfold increase in serum IgG titer over a 3- to 4-week period suggests recent infection. Detection of circulating IgM antibodies is consistent with recent infection but not clinical disease. A point-of-care assay for detection of canine distemper antibodies is available.* Vaccinated dogs, seropositive in this assay, probably do not need to be boosted. A presumptive diagnosis of distemper encephalitis can be made with increased CSF protein and leukocytes (lymphocytes predominating) plus a positive CSF antibody titer in a sample not contaminated with peripheral blood. Definitive diagnosis of canine distemper infection requires demonstration of viral inclusions by cytologic examination (see Color Plates 2D and 2E), direct fluorescent antibody staining of cytologic or histopathologic specimens, histopathologic evaluation, or PCR documentation of distemper viral DNA in peripheral blood, CSF, or conjunctival scrapings. (See Appendix I for availability of PCR testing.) Viral inclusions can rarely be found in erythrocytes, leukocytes, and leukocyte precursors of infected dogs. Inclusions are generally present for only 2 to 9 days after infection and therefore often are not present when clinical signs occur. Inclusions may be easier to find in smears made from buffy coats or bone marrow aspirates than those made from peripheral blood. Viral particles can be detected by immunofluorescence in cells from the tonsils, respiratory tree, urinary tract, conjunctival scrapings, and CSF for 5 to 21 days after infection.

*TiterCHEK CDV/CPV, Synbiotics Corp, San Diego, Calif.

Enteric Viruses

Indications • Viral enteritis induced by parvoviruses, coronaviruses, and other viruses should be suspected in young animals with fever and diarrhea, particularly if neutropenia is present (i.e., parvoviruses).

Analysis, Artifacts, and Interpretation • Determining serum antibodies to feline parvovirus and canine parvovirus or coronavirus is rarely performed clinically because positive results do not correlate with clinical disease. A point-of-care assay for detection of canine parvovirus antibodies is available.* Vaccinated dogs seropositive in this assay probably do not need to be boosted.

Detecting fecal shedding of canine parvovirus viral antigen by electron microscopy, virus isolation, fecal hemagglutination, fecal LA, or ELISA is more useful. In-office ELISA for canine parvovirus in feces seem to accurately detect fecal shedding of parvovirus in acute cases (see Chapter 9).†‡§ The specificity of the assays is good, but they cannot differentiate vaccine strains of parvovirus and virulent strains. False-negative reactions can occur. These assays may also detect feline parvovirus. Virus isolation or electron microscopy is required to identify other canine or feline viruses in feces.

Feline Infectious Peritonitis (FIP)

Rare Indications • FIP is an appropriate differential diagnosis in cats with fever; uveitis; retinal hemorrhage; nonseptic abdominal or

*TiterCHEK CDV/CPV, Synbiotics Corp, San Diego, Calif.
†ASSURE/Parvo canine parvovirus antigen test, Synbiotics Corp, San Diego, Calif.
‡Witness CPV canine parvovirus antigen test, Synbiotics Corp, San Diego, Calif.
§SNAP Parvo antigen test, IDEXX Corp, Portland, Me.

pleural exudates or modified transudates; anemia; hyperglobulinemia; and renal, hepatic, or neurologic abnormalities. Results of currently available tests cannot be used to definitely diagnose FIP.

Analysis, Artifacts, and Interpretation • Circulating antibodies against coronaviruses can be detected by IFA and ELISA in feline serum. Antibody to coronavirus indicates prior exposure to either enteric coronaviruses or FIP-inducing coronaviruses. A positive titer does not diagnose FIP or protect against disease. Feline vaccines containing bovine serum occasionally cause false-positive results. Cats with FIP can rarely have negative results because of rapidly progressive disease with a delayed rise in titer, disappearance of antibody in terminal stages of the disease, or immune complex formation. A positive coronavirus antibody titer does not predict whether a cat will ever develop FIP.

Titer magnitude cannot distinguish between exposure to enteric coronaviruses or FIP-inducing strains. Positive titers can be induced by vaccination for coronavirus. Kittens can be seropositive because of colostrum-derived antibodies until 9 weeks of age. If adult cats in the environment infect kittens, antibodies can be detected again 8 to 14 weeks later.

Current coronavirus infections can be detected by fecal virus isolation, electron microscopy of feces, or reverse transcriptase polymerase chain reaction (RT-PCR) of feces. However, positive test results do not indicate FIP because antibody-positive, healthy cats can pass coronaviruses (Addie and Jarrett, 1992). Definitive diagnosis of FIP requires histopathologic evaluation of tissues. Lesions visible by light microscopy are generally pathognomonic, but immunohistochemistry can be used to confirm coronavirus particles. PCR can also detect coronavirus particles in effusions, tissues, and blood (Barr, 1996). Detecting coronavirus by PCR in effusions and tissues predicts FIP, but detection in blood does not. Hyperproteinemia and polyclonal gammopathy (detected by electrophoresis; see Chapter 12) can occur, particularly in the noneffusive form. Monoclonal gammopathy rarely occurs. Classic nonseptic pyogranulomatous exudate or modified transudate with high protein and relatively low cell count (see Chapter 10) is commonly used for presumptive diagnosis. Electrophoresis can also be performed on body fluids. A gamma globulin fraction greater than or equal to 32% is highly suggestive of FIP, whereas an albumin:globulin ratio in body fluid greater than 0.81 probably rules out FIP (Shelly, Scarlett-Kranz, and Blue, 1998).

Feline Immunodeficiency Virus (FIV)

Common Indications • Cats with chronic weight loss, fever, rhinitis, conjunctivitis, gingivitis, dermatitis, diarrhea, uveitis, recurrent abscessation, clinical toxoplasmosis, any chronic infectious disease, chronic renal failure, or lymphadenomegaly should be evaluated for FIV infection.

Analysis, Artifacts, and Interpretation • IgG antibodies are detected in serum by ELISA, IFA, and Western blot immunoassay. Western blot immunoassay is performed in some commercial laboratories. An in-office ELISA is available for FIV antibodies and FeLV antigen combined.* Seroconversion occurs 5 to 9 weeks after inoculation in experimentally infected cats. Seropositive cats are probably infected with FIV for life. False-positive reactions can occur in the ELISA (Barr, 1996). Positive ELISA results should be confirmed via Western blot immunoassay or IFA. Finding circulating antibodies only confirms infection, not clinical illness. Kittens can have detectable colostrum-derived antibodies until 12 to 14 weeks. Because many clinical syndromes associated with FIV infection are caused by opportunistic infections, further diagnostic procedures may determine treatable causes. For example, many FIV-seropositive cats with endogenous uveitis are coinfected by *T. gondii*.

Virus isolation and PCR are available in some laboratories and can be used to confirm infection. A recently marketed FIV vaccine induces serum antibodies that are indistinguishable from antibodies induced by natural exposure, at least by use of currently available antibody tests. The ability of virus isolation or PCR to accurately differentiate naturally exposed and vaccinated cats is currently unknown.

Feline Leukemia Virus (FeLV)

Common Indications • Because of diverse manifestations of FeLV infection, testing is

*Snap FIV antibody/FeLV antigen Combo, IDEXX Corp, Portland, Me.

indicated in all clinically ill cats, especially those with evidence of infectious, neoplastic, reproductive, immunologic, or hematologic disease, as well as in clinically normal cats exposed to FeLV-positive cats.

Analysis, Artifacts, and Interpretation • Viral antigen (p27) is detected by IFA in neutrophils and platelets from blood or bone marrow, or in blood, plasma, serum, saliva, or tears by ELISA. Testing of serum gives the best results; tears and saliva should not be tested. Several point-of-care ELISA tests are available.*†‡§‖ Antibody titers to FeLV envelope antigens (neutralizing antibody) and against virus-transformed tumor cells (feline oncogenic cell membrane antigen, or FOCMA antibody are available in some laboratories, but the prognostic significance of the results is currently unknown; therefore, the tests are not recommended.

FeLV infection has six stages (Zenger and Wolf, 1992). Stages 1 through 3 are dissemination stages; bone marrow infection occurs in stage 4. Infected neutrophils and platelets are released from the bone marrow in stage 5, and virus appears in systemic epithelial tissues (including salivary glands and tear glands) during stage 6. ELISA can detect p27 antigen in serum during stages 2 through 6; p27 in cells is not detected by the IFA until stages 5 and 6. Thus serum ELISA is the first assay to become positive after infection, positive results occurring 2 to 30 weeks (generally 2 to 8 weeks) after infection.

Seropositivity can be detected by serum ELISA before a cat develops persistent (i.e., stages 4 to 6) infection; thus, some cats yield negative results after development of neutralizing antibodies. In healthy cats, seropositive results by serum ELISA should be confirmed by IFA or retesting by ELISA in 4 to 6 weeks. Some ELISA-positive cats reverting to negative status have become latently infected. The majority of latently infected cats are ELISA-negative on all testing, but virus can be isolated from bone marrow. Virus can also be localized to other tissues. False-positive ELISA results can be the result of poor laboratory technique.

A positive IFA test result has 99% correlation with virus isolation. False-negative reactions may occur when leukopenia or thrombocytopenia prevents evaluation of an adequate number of cells. False-positive reactions rarely occur from nonspecific staining of eosinophils. A positive IFA indicates that the cat is viremic and contagious. The viremia may be transient or sustained. Unless the IFA test is performed during a transient infection, the animal will likely (>95%) remain positive for life.

Virtually all IFA-positive cats are ELISA-positive. Finding an IFA-positive but ELISA-negative cat suggests technique-related artifact. A negative ELISA result is approximately 100% correlated with negative IFA and an inability to isolate FeLV. Cats that are ELISA-positive but IFA-negative are called *discordant*. Discordant results are usually caused by false-positive ELISA results, false-negative IFA results, or transient stage 2 to 3 infection. The American Association of Feline Practitioners has summarized testing recommendations for FeLV or FIV infections (http://www.aafponline.org/about/guidelines_retrovirus_testing_2001.PDF).

Some cats with latent infection localized to bone marrow have positive bone marrow IFA results. The most reliable means of identifying latent FeLV infections is virus isolation or PCR performed on bone marrow cells, but neither technique is widely available. A latently infected cat may become viremic (i.e., IFA- and ELISA-positive) after extreme stress or administration of glucocorticoids.

DIAGNOSIS OF DIROFILARIASIS *(DIROFILARIA IMMITIS)*

Cytology (Knott's Test or Filter Test)

Common Indications • Cytologic evaluation for microfilaria is indicated in dogs with signs consistent with heartworm disease (right-sided heart disease, coughing, dyspnea, eosinophilia, polyclonal hyperglobulinemia, protein-losing nephropathy [PLN]), in dogs about to begin prophylactic therapy (with diethylcarbamazine, ivermectin, or milbemycin), and rarely in cats with signs consistent with heartworm disease (i.e., dyspnea, cardiomegaly, unexplained vomiting).

Advantages • Very specific (microfilaria morphology differentiates *D. immitis* microfilaria

*Snap FIV antibody/FeLV antigen Combo, IDEXX Corp, Portland, Me.
†SNAP FeLV antigen test, IDEXX Corp, Portland, Me.
‡ASSURE/FeLV feline leukemia virus antigen test, Synbiotics Corp, San Diego, Calif.
§ViraCHEK/FeLV feline leukemia virus antigen test, Synbiotics Corp, San Diego, Calif.
‖Witness FeLV feline leukemia virus antigen test, Synbiotics Corp, San Diego, Calif.

from those of *Dipetalonema reconditum*), quick, and inexpensive; all concentration techniques (Knott's and filter tests) are much more sensitive than examination of fresh blood smears and are reasonably sensitive in dogs that have not been treated with filaricidal drugs.

Disadvantages • Up to 40% of dogs have spontaneous occult dirofilariasis (Zimmerman, 1992) and must be diagnosed by serologic testing and radiographic examination. All cytology tests have poor sensitivity in cats.

Analysis, Artifacts, and Interpretation • A positive test result diagnoses heartworm disease, except in juveniles less than 4 to 5 months of age that could have received the microfilaria by transplacental transfer. Up to 40% of infected dogs are amicrofilaremic. Infected dogs receiving ivermectin or milbemycin as heartworm preventive are commonly amicrofilaremic. Clinical or laboratory signs of heartworm disease despite one or more negative microfilaria tests indicate serologic testing for circulating heartworm antigen, chest radiographs, echocardiography, or a combination of these.

Heartworm Adult Antigen Titer

Common Indications • Amicrofilaremic dogs or cats with clinical signs, laboratory abnormalities, or thoracic radiographic changes consistent with dirofilariasis. Dogs on ivermectin, selamectin, or milbemycin preventive can have sterile female adult worms and be amicrofilaremic. The test can also be used to assess efficacy of adulticide treatment.

Advantages • Greater sensitivity when compared with microfilaria detection techniques.

Disadvantage • More expensive than microfilaria detection techniques.

Analysis, Artifacts, and Interpretation • ELISA can detect circulating heartworm antigen in serum; several kits are commercially available.*†‡§‖¶ Intestinal parasites, *D. reconditum*, hemolysis, and concurrent use of diethylcarbamazine, ivermectin, or milbemycin do not alter results of antigen assays. In dogs, *D. immitis* antigen tests may be positive as early as 5 to 6 months and are usually positive 6 to 7 months after infection. False-negative results usually occur in early stages of infection and may occur in single-sex infections (male only) or in animals with low worm burdens (< 3 to 5 worms). Retesting in 2 to 3 months should be performed to detect dogs in which results were negative in early stages of infection. After successful adulticide treatment, test results become negative in approximately 12 weeks. In experimental infections, cats testing positive did so about 8 months after infection. However, single sex or low worm burden infections can lead to false-negative results. Therefore a positive antigen test result is specific for infection, but a negative result does not rule out dirofilariasis. In cats, the combination of serum antigen test results with serum antibody test results is more sensitive than performing either test alone (Synder et al, 2000).

Definitive diagnosis requires detecting circulating microfilariae, characteristic radiographic signs (i.e., right-sided cardiac enlargement, increased diameter with or without tortuosity of pulmonary arteries, pulmonary interstitial infiltrate), or circulating heartworm antigen. Amicrofilaremic dogs with clinical signs of disease should be evaluated for serum antigen and with thoracic radiographs. In a previously infected and treated dog, it may be impossible to differentiate "new" from "old" radiographic lesions. In such cases, serum antigen testing is indicated.

Heartworm Antibody Titer (Feline)

Rare Indications • Coughing, unexplained vomiting, syncope, or radiographic evidence of heartworm disease.

Analysis, Artifacts, and Interpretation • Several ELISAs detect antibodies to *D. immitis* in feline sera.**††‡‡ The assays are more sensitive

*Witness HW heartworm antigen test, Synbiotics Corp, San Diego, Calif.
†DiroCHEK HW heartworm antigen test, Synbiotics Corp, San Diego, Calif.
‡SNAP heartworm antigen test, IDEXX Corp, Portland, Me.
§PetChek heartworm PF antigen test, IDEXX Corp, Portland, Me.
‖Solo Step CH test cassettes heartworm antigen test, Heska Corp, Fort Collins, Colo.
¶Solo Step CH test strips heartworm antigen test, Heska Corp, Fort Collins, Colo.
**Witness FHW feline heartworm antibody test, Synbiotics Corp, San Diego, Calif.
††ASSURE/FH feline heartworm antibody test, Synbiotics Corp, San Diego, Calif.
‡‡Solo Step FH test cassettes heartworm antibody test, Heska Corp, Fort Collins, Colo.

than microfilaria demonstration techniques. The assays are very specific; no cross-reactivity exists with *D. reconditum*. The positive predictive value for heartworm disease is less than 100%, however, because circulating antibodies can be present in cats that have cleared the infection naturally. False-negative antibody test results also occur; therefore, serum antibody and antigen tests should be performed in concert in cats with suspected dirofilariasis (Synder et al, 2000).

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16

Harold Tvedten and Rick L. Cowell

Cytology of Neoplastic and Inflammatory Masses

Cytologic diagnosis has many advantages. Minimal equipment is needed, making cytology possible in any practice. A nearly immediate cytologic conclusion eliminates waiting one or more days for a histologic diagnosis. Fine-needle aspirates are minimally invasive and minimally stressful for patients. Cytology may save the expense of anesthesia and surgery. This chapter discusses cytologic evaluation of masses. Cytologic analysis of fluid, vaginal secretions, semen, urine sediment, respiratory tract material, and specific organs is described in the appropriate chapters.

Cytology can alter or eliminate surgery. Obviously benign masses, such as lipomas and epidermal inclusion cysts, need not be

removed immediately. If they are removed, a close resection (i.e., "shelling it out") is adequate, whereas wide excision is indicated for potentially malignant neoplasms. Any cytologic evidence of malignancy (e.g., possible carcinoma or sarcoma) dictates a search for metastasis before surgical removal and histologic diagnosis. Evidence of metastasis is a contraindication for surgical removal. Neoplasms that are too vascular or invasive for surgery may require cytology for diagnosis instead of histopathology. If a specific tumor diagnosis is made, specific treatment may be started early. Diagnosis of a systemic neoplasm like lymphoma usually indicates chemotherapy rather than surgery.

Cytology has limitations. Histopathology is usually more diagnostic and definitive, because more information (i.e., tissue architecture) is available from a histologic section than from the small number of cells on a cytologic smear. Although cytology gives a definitive diagnosis in many cases (e.g., infectious diseases, mast cell tumor, lymphoma, lipoma), cytology often gives only a general diagnosis (e.g., epithelial mass without cytologic evidence of malignancy), requiring histologic evaluation for a definitive diagnosis. Of 147 skin tumors, only 105 (71%) cytologic diagnoses agreed with histologic diagnoses (Griffiths, Lumsden, and Valli, 1984). Radical steps (e.g., euthanasia) should await confirmed diagnosis when possible.

Exceptions exist in which cytology is as diagnostic (or more diagnostic) than histology (e.g., individual cell detail of leukemias is more diagnostic than tissue patterns). Cytology was correct in 60 of 64 round cell tumors and occasionally more diagnostic than histology (Duncan and Prasse, 1979). Cytologic and histologic diagnoses agreed on all of the following tumors: 37 mast cell tumors, 11 melanomas, 2 histiocytomas, and 1 cutaneous lymphoma (Griffiths, Lumsden, and Valli, 1984). Accurate cytologic diagnoses were also made for squamous cell carcinomas, lipomas, and metastasis to lymph nodes.

CYTOLOGIC TECHNIQUES

Slide Preparation

The major goal is to obtain a significant number of well-stained, intact cells reflecting the composition of the mass. One should consider the site being sampled. An aspirate or impression smear should reflect the primary disease. Samples of a body surface over a mass often have only pus or necrotic or reactive cells, so they are often misleading (Figure 16-1). Samples of deeper tissue are more likely to be diagnostic. Epithelial cells at the edge of ulcers appear anaplastic, because they are actively proliferating to cover the ulcer (e.g., corneal ulcers can cytologically resemble squamous cell carcinoma). Ulcerated surfaces often have secondary inflammatory and septic changes. Malignant-appearing cells from deep under a surface are much more likely to truly indicate that the mass was malignant. Soft centers of a mass may be necrotic or hemorrhagic, so a smaller firm mass or a more viable-appearing area at the edge of a mass may be more diagnostic.

A fine-needle aspirate need obtain only one or more small drops of fluid to streak out similar to a blood smear. Too much aspiration can cause bleeding and dilution of the sample. One should use a 5 to 10 ml syringe and 22- to 20-gauge needle. Vacuum should be applied to the syringe after the needle has penetrated the mass, and the vacuum should be maintained while one passes the needle tip back and forth through the mass; then the vacuum should be released and the

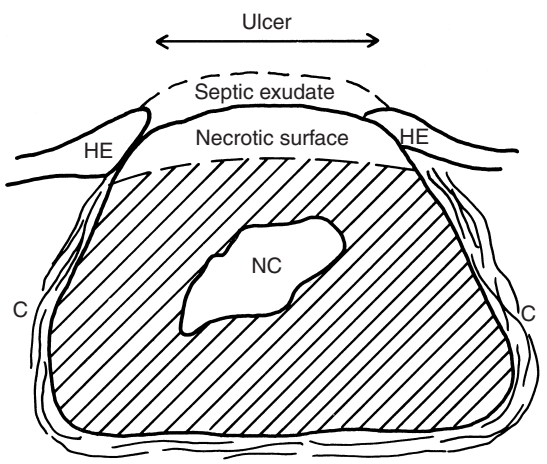

FIGURE 16-1. Sampling sites from a mass. Different cell populations are retrieved from different sites. Cells from the proliferative mass *(hatched area)* are representative and diagnostic. Samples from the surface may be only necrotic material or septic exudate. Immature-appearing hyperplastic epithelial cells *(HE)* from the edge of an ulcer may mimic a carcinoma. Material from a necrotic center *(NC)* may resemble only debris. Cells from the boundary *(C)* may be fibroblasts from the capsule or a local inflammatory response. Mature fat or blood may be collected from adjacent normal tissue.

needle withdrawn. Next, the clinician should remove the needle from the syringe and fill the syringe with air. The needle should then be reapplied, and one should gently blow a drop of fluid on a glass slide. Finally, the fluid should be drawn out to a smear with coverslip or another slide. With very fragile cells (e.g., lymphoid, necrotic), one should use air blown from the syringe to blow out the drop to a thin smear.

The smear must have a thin area where cells may spread out flatly and expose a large surface area to view (Figure 16-2). If the smear is thick, cells are supported more upright on the slide and have a smaller diameter. Cells in thick areas are taller and thus stain darker. Proteinaceous and necrotic debris in dried fluid surrounding cells interferes with staining. If fluid is viscous, a squash preparation may be necessary to get a thin smear. A drop of fluid is placed between two slides; while the drop spreads to its maximum diameter, the slides are slid apart while their surfaces are kept pressed together. This creates a smear on each slide. Lymphoid cells are particularly fragile and should be handled gently.

A nonaspirate (capillary) technique has been described (Menard and Papapeorges, 1995). This technique is reported to be easier to perform and to yield less bloody samples of equal or greater cellularity, especially from highly vascular tissues (e.g., liver aspirates). The technique described here is a modification of the nonaspiration technique used at Oklahoma State University. Air (i.e., 5 ml) is aspirated into a 10 ml syringe, and a 22-gauge needle is attached. The syringe is held at the base of the needle to allow for better control, and the mass is stabilized with the operator's free hand. The needle is introduced into the mass and rapidly moved back and forth along the same tract five or six times. Negative pressure (i.e., aspiration) is *not* applied. The cells are collected by shearing and capillary action. The needle is withdrawn from the mass, and collected cells are quickly expelled onto a clean glass slide by depressing the plunger. The collected material is then spread as discussed earlier.

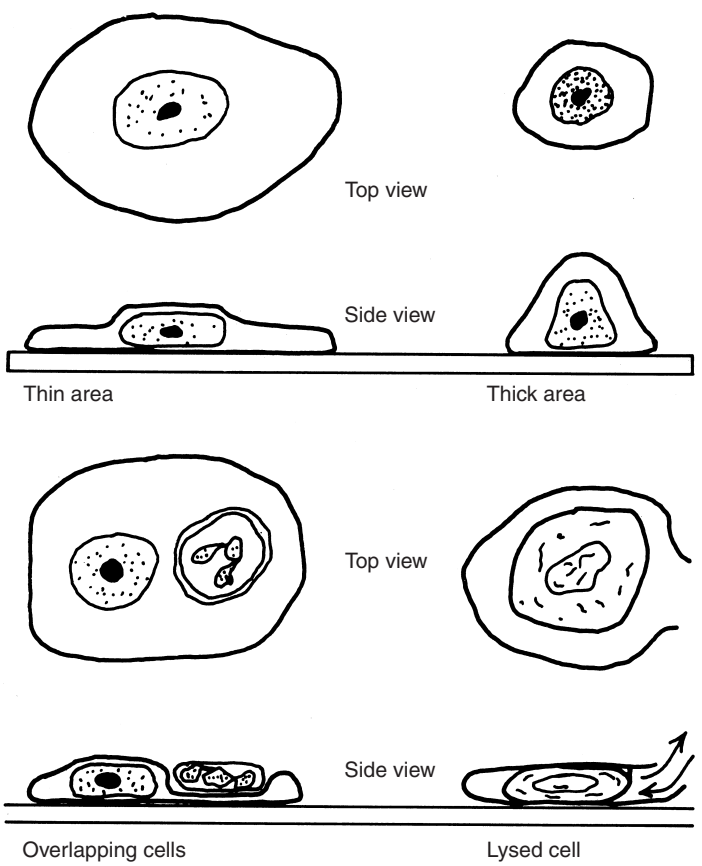

FIGURE 16-2. Cell morphology in three dimensions. The cell *(upper right)* is in the thick part of a smear, so it fails to spread out over a large surface area. It appears smaller and darker from the top compared with the cell on the upper left, which has spread out in a thin area of a smear, allowing proper evaluation. The epithelial cell *(lower left)* has a neutrophil dimpled into its surface. The neutrophil appears to be in the cell when viewed from above. The partially lysed cell *(lower right)* has a swollen, enlarged nucleus and nucleolus, which may appear malignant instead of only damaged.

Generally, only one smear can be made from each collection attempt. Therefore three or four collections from different sites should be taken.

For impression smears, a freshly cut surface of a representative area of the specimen is gently touched to several areas of glass slides without any twisting or rubbing motion. Excess fluid may be first removed by blotting the tissue with a paper towel. One should check to see if cells exfoliated well before placing the tissue in formalin. Fibrous masses usually do not release cells easily and may need to be scraped with a scalpel to obtain enough cells for diagnosis. The moist material on the blade is streaked on slides.

Patient identification and the date *must* be noted on the slide. A site description is needed to interpret the results, especially if multiple sites are sampled. Mailing containers for slides should be too large (e.g., small box) to fit through the post office's automatic stamp canceling machine. Thin two-slide cardboard boxes that fit in an envelope often arrive with crushed glass slides, even when they are marked "Hand Cancel."

NOTE: Postal machines often crush slides mailed in thin, flat containers.

Smears are air dried for Wright's-type stains and new methylene blue (NMB) staining. Alcohol fixation is required for Papanicolaou's (Pap's) or Sano's stain. Air drying cells slowly in a moist environment may cause cell distortion. Although rarely used, a hair dryer speeds drying. One should hold the dryer far enough away from the smears to avoid "cooking" cells; during storage, exposure to dust, molds, and pollen should be prevented. One should remember that flies eat unstained cells and that sides should only be touched on the slides, because squamous cells from fingerprints can contaminate smears and interfere with interpretation.

Formalin exposure is a common problem causing excessive blue staining of smears stained with Wright's stain. Tissue used for impression smears should not have been placed in formalin before the slides were made. Formalin should not be stored near the stains or smears. Formalin should not be submitted in the same package with cytologic smears, where the fumes may act on unstained cells. Heparin anticoagulant also causes a blue discoloration of Wright's-stained cells.

NOTE: Formalin causes poor cell staining, so cytology slides and formalin-fixed histopathology samples should not be mailed in the same package.

Stains

Most practices need only a modified Wright's-type stain and NMB. The "Wright's" stains and "Quick" stains used today are not the original Wright's or Giemsa (i.e., Romanowsky's) stains but have blue and red dyes to give similar staining characteristics. The dyes stain acidic structures such as nucleic acids (deoxyribonucleic acid [DNA] and ribonucleic [RNA]) in nuclei blue to purple and basic structures such as proteins (e.g., hemoglobin in erythrocytes) red to orange. Changes in pH in rinse water or the sample can affect staining characteristics. Diff-Quik stain is a popular, because one can easily adjust the color of the cells. The blue and red dyes are in separate jars; by increasing or decreasing the number of times one dips the slide in a color, the intensity of blue or red is increased or decreased. A Diff-Quik staining kit from VWR Scientific Products (68100-408) was priced at $149.04, and a 4 oz bottle of NMB cost $8.00 in 1998. The Diff-Quik stain illustrates distemper inclusion bodies well on blood smears. Modified Wright's preparations occasionally fail to stain granules in some mast cells and basophils.

NMB is a monochrome stain with variably intense blue staining. One uses it as a wet mount by placing a drop of NMB on an air-dried smear and applying a coverslip. To prevent retention of an air bubble over the most diagnostic part of the smear, the coverslip should be used to gently pull the drop of NMB over the cells to moisten them before slowly applying the coverslip. Staining is immediate. NMB stains nuclear material well and demonstrates distinct chromatin patterns (see Color Plate 6C). This nuclear detail is useful in evaluating malignant criteria. The transparent nature of NMB is a major advantage, because one can focus the microscope through different depths of tissue fragments to judge individual cell detail and architectural patterns in three dimensions. Tissue fragments are often the most diagnostic material on smears from neoplasms but stain too darkly to evaluate with Wright's stain. The fragments are like tiny biopsy sections that allow evaluation of how cells were oriented in the mass. These architectural patterns help identify the tissue type. One can evaluate adjacent cells for true

variability suggesting malignancy, compared with the variability of isolated cells on a smear that may have come from different areas or cell types in the mass.

In summary, NMB is excellent for nuclear detail, tissue fragments, and most fungi. Wright's stain is best for inflammatory lesions, because the stained appearance of leukocytes (white blood cells [WBCs]) is similar to that in blood smears and bacteria are stained a characteristic, variable blue color (see Color Plate 4A). Although Wright's stain is not as good as NMB for nuclear detail, it is acceptable for evaluating tissue cells for criteria of malignancy and excellent for most fungi. Use of both Wright's and NMB stains for the same lesion is best, because different characteristics of the cells are illustrated by each stain. Cytoplasmic structures often show best on Wright's stain. Organisms not prominent on one stain usually are visible on the other. Use of only one stain may contribute to missing a diagnostic feature.

Other stains may be used. A combination of a drop of NMB and Sudan stain or other neutral fat stain selectively stains lipid and gives adequate cell detail. This stain combination is useful for fatty livers (see Color Plate 5E), chylothorax (see Color Plate 5B), aspiration pneumonia, or lipid granulomas. NMB stains the nucleus and other cell structures blue, and the Sudan stains lipid red.

Gram's stain differentiates gram-negative from gram-positive bacteria well on cultures from blood agar plates. Different antibiotics are used for gram-positive versus gram-negative bacteria. Gram's staining is inconsistent for bacteria in exudates, however, and caution is suggested because staining varies. In thick smears the bacteria may not decolorize, creating a false impression that the bacteria is gram positive. In thin smears, bacteria may decolorize too much. Gram staining is not sensitive for screening cytologic smears for gram-negative (i.e., red) bacteria in low numbers in a red, proteinaceous background (see Color Plate 4C). Wright's stain consistently stains bacteria blue, allowing easier detection and definition of size and shape. An exception is mycobacteria that do not stain at all because of their waxy coat. Gram's stain is best used in bacteriology laboratories, whereas a Wright's-type stain is preferred for routine cytology. Acid-fast stain is rarely needed, because mycobacterial infections are uncommon (see Color Plate 4D).

Pap's stain or a modified Sano's trichrome stain is routinely used in human medicine and rarely in veterinary practices. This stain has advantages, the authors' did not find it cost-effective or necessary. Specific malignant criteria have been established for cells stained with these stains. Pap's stain is a transparent stain that permits evaluation of thick tissue fragments and fine evaluation of nuclear characteristics. Smears need to be immediately fixed in alcohol before Pap's staining.

Microscopes

A good quality, well-maintained microscope is needed for cytology. A binocular microscope is more comfortable for long viewing periods. Four objectives are recommended: One should use 4x and 10x for scanning a smear and 50x oil and 100x oil for fine detail. Objectives of similar magnification may be substituted. A properly equipped, good-quality microscope will cost at least $1200 to $2000 but will have multiple uses in a clinic and should last a lifetime.

A 50x oil plan achromatic objective (i.e., 40x to 60x oil) pays for itself ($350 to $1,000) by the time it saves. Magnification is sufficient for most detail, and more cells can be seen in a shorter time. Having more cells in the larger field of view than 100x allows better comparison of variations among cells and easier identification. The whole field of view is in focus with the more expensive planachromatic lenses, whereas the perimeter is out of focus with cheaper achromatic lenses. With a wider field of view, less time is needed to find abnormal cells. A 50x oil objective avoids the need to coverslip smears.

Wright's-stained cells observed with a 40x high-dry objective appear fuzzy, because the cells are surrounded by air. Using mounting media with a coverslip or oil (with a coverslip) eliminates the air and cell interface and allows good cellular detail with a high-dry lens. Adding a drop of oil is much faster than permanently coverslipping smears. Oily smears are messy to store and less permanent, however.

The microscope should be cleaned and lubricated by a trained repair person every 1 to 3 years. Complete covering of the scope prevents accumulation of dust in hard to clean places. Oil should be removed from lenses at the end of a work period, because oil can penetrate behind some lenses to render them useless. Debris that hardens on lenses may require xylene or lens cleaner to remove. Kimwipes are lint-free tissues that are

satisfactory for cleaning microscope objectives not used for photomicroscopy. Kimwipes absorb oil better than does lens paper and thus clean more effectively. Concave lenses require a cotton-tipped swab moistened with lens cleaner to clean the recessed area. Final polishing of the objective lens should be with lens paper. One should clean filters, light sources, stage, and condenser as needed; sharp vibrations (e.g., dragging the microscope along the surface of a desk, setting it down hard) should be avoided. This can knock the prism out of alignment and cause a double image.

The condenser must be in the proper position for optimal detail (e.g., for finding small bacteria). To position the condenser near the optimal setting (i.e., Köhler's illumination), it should be close beneath the glass slide on the stage. More specifically, several simple steps should be performed daily. While at high power (e.g., 40x objective), one should focus on the cells on a smear. The field diaphragm at the bottom of the microscope should be completely closed, and the condenser should be moved slightly up or down until the circle of light in the field has sharp edges. The condenser should be left at this optimal height. One should then center the circle of light to the center of the field and open the field diaphragm so that no shadow appears in the field. The diaphragm should then be closed in the condenser (from fully open) until one gets the first hint of slightly decreased light; this should give slightly better contrast to intracellular structures. This adjustment to the condenser's diaphragm may also be done by removing the right 10x eyepiece and looking down the tube as one closes the condenser's diaphragm from the fully open position to a slight decrease (i.e., 5% to 10% decrease) in the diameter of the light. The aperture diaphragm in the condenser is often closed too much (it is better to leave it wide open than get less contrast by closing it too much). When optimal positioning is achieved, any dust on the condenser or light source is in view. One should clean the dust away or slightly move the condenser so that the dust cannot be seen.

Some people avoid the optimal position if the light appears too bright, but it is better to use a neutral-density filter, a dimmer bulb, or a more controllable light source than to accept poor cell detail. The "swing-in" condenser lens on Reichert (American Optical) microscopes should be swung out, except at low power (i.e., 2.5x and 4x objectives). When morphologic detail is not needed and the viewer wants only to easily find objects such as parasite ova, urinary casts, or platelets in a hemocytometer, the condenser is moved to the lowest position. This position gives structures more contrast.

Some common problems with microscopy include placing the slide upside down. In this case one can focus on cells at medium magnification (i.e., 10x, 40x) but not with the 100x oil objective, because cells are on the underside of the slide. If the coverslip or mounting medium is too thick, one also cannot focus at high magnifications. If the fine focus will not turn any farther in the direction needed, one should adjust the coarse adjustment past the plane of focus needed, then turn the fine focus knob in the opposite direction to regain focusing ability. If the cells look refractile and have poor detail, the lighting is probably wrong. In this case one should adjust the microscope to Köhler illumination. If cells are in focus with 100x objective but not the high-dry 45x objective, the 45x objective is contaminated with oil and must be cleaned with a cotton swab.

CYTOLOGIC CONCLUSIONS

The usual composition of a mass is a proliferation of tissue cells, an accumulation of inflammatory cells, or both. Miscellaneous masses include hematomas, cysts, or focal areas of necrosis. Characteristics of each type and its variants follow and are briefly summarized in Figure 16-3.

Slide Reading Approach

Veterinarians have ample microscopy, histology, and pathology training, so most can learn to read cytologic smears as long as they recognize their limitations and constantly learn from their cases. Cytologic evaluation may be performed on excised masses and then the descriptions and conclusions may be compared with histopathology reports. Cytologic evaluation is a visual task, so one should obtain one or more cytologic atlases, books, or CD-ROMs for visual comparisons (Raskin and Meyer, 2001; Baker and Lumsden, 2000; Cowell and Tyler, 1989; Perman, Alsaker, and Riss, 1979; Tvedten, 2000). An organized approach to an aspirate or impression smear of an abnormal mass is necessary for

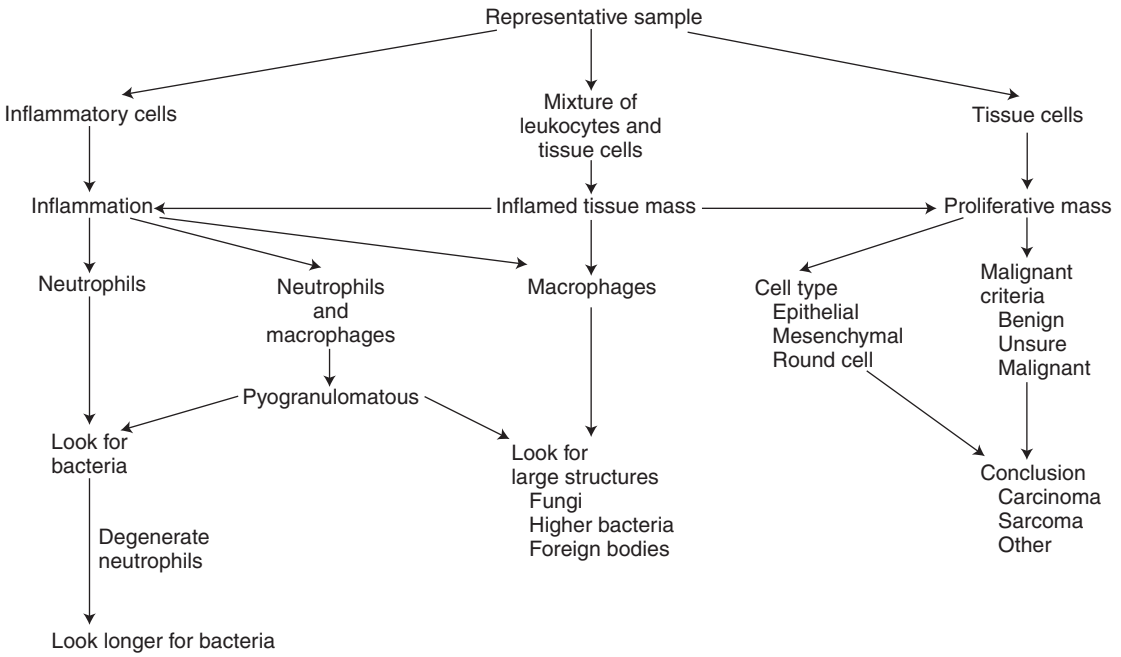

FIGURE 16-3. General cytologic approach. Most diagnostic samples are initially divided into an inflammatory or a proliferative pattern or both. Inflammatory samples are examined for likely causative organisms. Degenerative changes in neutrophils stimulate a longer search for bacteria. Proliferative patterns are evaluated for cell type and evidence of malignancy. Summary statements are then made. With an inflamed tissue mass, one pursues an inflammation approach or a proliferative mass approach, depending on the predominant cell type or clinical intuition.

consistent conclusions. A summary of steps follows, and details are provided in later discussions.

The cytologic specimen may not represent the lesion. Adjacent structures may be sampled inadvertently. For example, a common error is to aspirate the submandibular salivary gland instead of lymph node. Smears have normal, mature acinar and ductal structures with foamy epithelial cells. The conclusion was that the sample was not representative, not that an adenoma or metastatic carcinoma was present. Other examples include inadvertently sampling the liver while obtaining "thoracic" aspirates and having the needle pass all the way through a small mass and to only aspirate subcutaneous (SC) fat. One may contaminate a cystocentesis urine sample with gut bacteria by inadvertently puncturing the intestine. Correct conclusions often require intuition and experience.

1. First establish that a sufficient number of intact, properly stained cells are present and properly represent the mass.
2. Scan smears at low power to determine variation in distribution and content. Look for large structures (e.g., fungus, bacterial colony, parasite eggs, or larvae).
3. Begin fine evaluation of cells in an area with intact cells of good staining quality.
4. Determine whether the cell population is primarily inflammatory. If so, attempt to identify the etiologic agent.
5. Determine whether enough tissue cells of one type are present to indicate a noninflammatory, proliferative tissue mass.
6. With proliferative tissue masses (e.g., neoplasia or hyperplasia), determine cell types (round, spindle, epithelial) present and amount of evidence indicating malignancy.

The initial effort should be to screen smears grossly for those areas most likely to be diagnostic. In tumor cytology, the slides with small tissue fragments are most promising and should be stained with NMB or other semitransparent stain. One should begin with the smears that are dark blue. The most cell-rich smears are most likely diagnostic and stain bluest, because they have the most nuclei. Hemodiluted smears with few nucleated cells appear orange with Wright's stain, suggesting reduced chance of diagnosis.

Too often one goes too quickly to the 100x oil objective and stays at that power until fatigued. The scanning power must be used first, and often, to locate productive areas of the smear, which are then evaluated with an oil objective power. Promising areas are thin and have intact, well-stained individualized cells. Cells poorly stained with Wright's stain have an altered, pale, diffuse, blue color. The color and streaked out appearance of necrotic, lysed cells indicates an area to avoid. Diagnostic structures (e.g., bacterial and fungal colonies) may be rare and isolated, so one should take time to scan smears and not spend all the time at high magnification in a few areas. Similarly, tissue fragments that have the valuable architectural patterns are irregularly distributed and found by scanning.

One should determine and record the cell population in terms of percentage of various cell types. A hematology differential counter is useful to keep track of various cell types. An accurate and complete description makes conclusions easier. Performing a differential count forces one to try to identify all cells and not be biased by prominent cells such as eosinophils, plasma cells, and large cells that seem more numerous than small cells such as lymphocytes. One should not expect to identify all cells. Experts often use a category of "other" cells for unidentified cells and then morphologically describe the cells and suggest their origin. If the smears are moderately to markedly cellular and 99% of the cells are WBC, including 90% neutrophils, the conclusion is obviously that the mass is inflamed, perhaps an abscess. If only 1% are large, malignant-appearing mesenchymal cells, they would most likely be reactive cells (e.g., fibroblasts and lymphoblasts or monoblasts in an inflamed mass), not a sarcoma. It is common to have a few unidentified cells in inflammatory masses that have cytologic characteristics of malignancy. If they are few, they are ignored. On the contrary, if 25% to 50% or more of the cells are spindle shaped mesenchymal cells, the conclusion would be that the mass is a connective tissue proliferation.

If evidence indicates a neutrophilic inflammatory mass, one should look for the most likely causative agent (i.e., bacteria). Other inflammatory patterns suggest other causes. If the intermediate conclusion is that the mass is a connective tissue proliferation, one should consider the variety and magnitude of the malignant criteria. The amount of cytologic evidence of malignancy is then used to differentiate a sarcoma from a benign proliferation, such as a fibroma or active granulation tissue.

Inflammatory Masses

Inflammation is diagnosed much more frequently with cytology than is neoplasia. Cytologic diagnosis simply requires an adequate number of inflammatory cells. The number of cells sufficient for diagnosis varies with the sample. A rare plasma cell and phagocytic macrophage aspirated from the eye indicates inflammation, whereas thousands of neutrophils are found in pus. In hemodiluted samples, one considers the number and type of WBC usually found in blood. Blood has about a 500 : 1 RBC : WBC ratio, with mainly neutrophils and lymphocytes. More WBC (e.g., 20 : 1 ratio) or the presence of WBC not found in blood (e.g., plasma cells, phagocytic macrophages) must be present on hemodiluted samples to diagnose inflammation. Based on predominant WBC type, different terms are used and different causes are suspected.

> NOTE: Inflammation is much more commonly diagnosed by cytology than neoplasia.

Neutrophilic Inflammation

Neutrophilic infiltrates (e.g., exudation, suppuration, abscess formation, purulent inflammation) are so frequent they are almost synonymous with inflammation. Neutrophils are the most motile WBC and the first to infiltrate an area. Some call neutrophilic inflammation "acute inflammation," even though neutrophils may be prominent in chronic but active inflammation. Therefore the term *acute* may refer to a cell type (e.g., predominance of neutrophils) and not always to a time interval. Pus is composed mainly of neutrophils in a loose, fluid matrix of proteinaceous cell debris; therefore these cells are most easily obtained for an aspirate or impression smear. Other cells in an inflammatory mass may not exfoliate as easily (especially fibroblasts), if scarring is present. A neutrophil migrating between stratified squamous epithelial cells may indent into the surface of a squamous cell and appear as if it is in the squamous cell when it exfoliates (see Figure 16-2). Neutrophils are associated with bacterial infections and some yeast infections (e.g., *Candida*), but nonseptic causes include immune-mediated processes

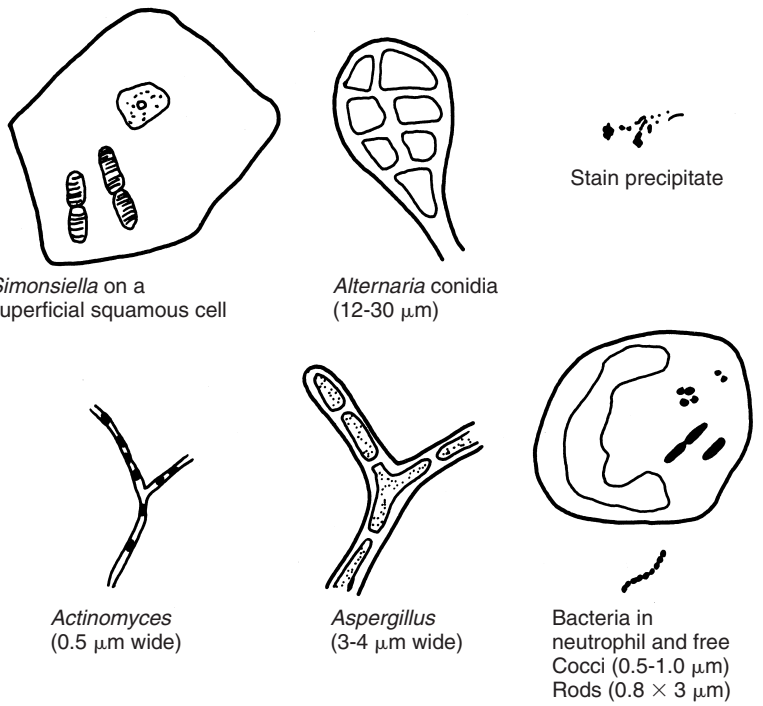

FIGURE 16-4. Miscellaneous cytologic structures. The top row includes nonpathologic structures potentially confused with causative agents. *Simonsiella (illustrated as two pairs of large, flat, oval bacteria that resemble footprints)*, is normal flora of the oral cavity. *Alternaria* is composed of septate hyphae and is a common contaminant. Its conidia are club-shaped with chambers that may be mistaken for *Microsporum*. Stain precipitate is more irregular in shape and size than the bacteria illustrated below it. *Actinomyces* is much narrower and lacks the distinct cell walls of fungal hyphae, such as *Aspergillus*. Bacteria are best identified in the clear cytoplasm of neutrophils. Distinct cocci or rods forming pairs, tetrads, or chains are unlikely to be artifacts.

(e.g., lupus polyarthritis) and chemical irritation (e.g., bile peritonitis).

Bacterial Sepsis • Neutrophilic inflammation indicates a search for bacteria. The best place to search is in cytoplasm of neutrophils (see Color Plate 4A). The neutrophils' cytoplasm is usually clear and free of granular debris. Macrophages often contain phagocytized cell debris that can mimic bacteria. Bacteria are more prominent in the clear neutrophilic cytoplasm, and the phagocytic vacuole may help outline the organism.

NOTE: Bacterial infection is best shown by finding bacteria inside neutrophils. Moderate to many free bacteria in fresh samples of an exudate also indicates infection. Infection is not, however, ruled out by a lack of bacteria in cytologic samples with neutrophilic inflammation, because bacterial numbers may be below detectable levels of a microscopic examination.

Bacteria have uniform shapes and sizes, in contrast with granular debris. Formation of uniform pairs, tetrads, and chains identifies structures as bacteria. Wright's stain precipitate is coccoid in appearance and may mimic coccoid bacteria. Irregular size of the precipitate, a more purple color than the blue of bacteria, and a refractile appearance differentiate stain precipitate from bacteria (Figure 16-4).

The description of "bacteria" should include number, location (e.g., free in the smear, phagocytized, or both), appearance, and whether a pure or mixed population is present. These observations permit certain conclusions. For example, a pure population of large cocci in pairs and tetrads within neutrophils from an abscessed lymph node suggests an infection (e.g., *Staphylococcus*), whereas a mixed population of variably sized, large rods and cocci in neutrophils in abdominal fluid suggests a ruptured gut. Beaded filamentous organisms indicate higher bacteria (e.g., *Actinomyces*) (see Color Plates 4B and 4C).

Phagocytosis of bacteria is a better indication that there was bacterial infection than are free bacteria. Even free bacteria in a neutrophilic exudate supports infection, especially if numerous in a fresh sample. Bacteria free in the background may have been bacterial or fungal contamination from the stain or from improper sample handling, especially in samples that were wet (e.g., tracheal wash) for hours or days before a smear was made. Bacteria on stratified squamous epithelial cells are usually normal flora from a body surface. A normal flora of the oropharyngeal area of dogs is *Simonsiella*. Finding this unique, huge

bacterium indicates at least part of the sample came from the mouth or pharynx (see Figure 11-10 and Figure 16-4). Finding *Simonsiella* and other bacteria on squames suggests that a few, free bacteria found on a transtracheal wash or bronchoalveolar lavage may not have originated from the lower respiratory tract.

Degenerative Neutrophils • How long should one search for bacteria in neutrophilic exudate? Bacteria will not be in every field if low numbers were in the lesion. Antibiotic treatment may reduce bacterial counts below levels detectable cytologically. A useful key is appearance of neutrophils. One should search for bacteria longer than usual if neutrophils appear degenerate. Bacterial toxins cause rapid neutrophil death (karyolysis). Degenerative changes suggest but do not prove sepsis. A predominance of nondegenerate neutrophils suggests a nonseptic environment and indicates a shorter (i.e., 5 to 10 minute) search for bacteria. Some bacteria seem less toxic to neutrophils, however, and bacteria occasionally are found in nondegenerate neutrophils (see Color Plate 4A).

Morphologically degenerate neutrophils are characterized by swelling of the nucleus (karyolysis) and cytoplasm. Karyolysis appears as a wider, more irregularly shaped, lighter-staining nucleus lacking the dark, distinctly granular chromatin pattern and thin lobulated shape of viable nuclei (see Color Plate 4B). Severely degenerate neutrophils may hardly resemble neutrophils as they swell and lyse into "globs" of nuclear debris. Degenerative changes caused by bacteria must be differentiated from partial lysis because of sample storage, trauma to fragile cells during streaking of the smear, or nonbacterial toxic effects (e.g., urine). Inexperienced cytologists tend to overidentify degenerative neutrophil changes by examining partially lysed cells. Pus always has a variable number of damaged neutrophils. Evaluate only intact, undamaged cells. If the neutrophils with intact cell boundaries appear nondegenerate, lysed neutrophils on the slide are probably artifactually broken rather than degenerate from bacterial toxins.

Nondegenerate neutrophils resemble normal cells visible in blood smears (i.e., clear cytoplasm; a dark, thin, lobulated nucleus). A lack of bacterial toxins permits cells to live longer. Old neutrophils become hypersegmented and are evidence of a nontoxic environment. Nondegenerate neutrophils dying slowly of old age also have pyknotic or karyorrhectic nuclei, which appear as dense, dark, purple-staining round masses in cells or free in the background. Incidentally, karyorrhectic nuclear material may mimic yeast.

Necrosis • Viscous necrotic material is abundant in pus. Streaks of nuclear debris must not be misidentified as mucus or fungal hyphae. Being able to trace the streaks back to a partially lysed nucleus identifies them as nuclear debris. Necrosis is suggested by numerous lysed cells but is proved by cellular debris in macrophage vacuoles. Cholesterol crystals reflect breakdown of certain lipids and appear like panes of glass (i.e., clear rectangular crystals). The crystals are unstained and only outlined by other material taking the stain. Blood pigments and crystals occur with RBC necrosis.

Granulomatous and Pyogranulomatous Inflammation

When the inflammatory population is mainly macrophages (granulomatous) or a mixture of neutrophils and macrophages (pyogranulomatous), one must consider a causative agent larger than bacteria (e.g., fungus, foreign body). If an agent is not found, only a morphologic conclusion of pyogranulomatous or granulomatous inflammation is commonly reached. Neutrophils exfoliate more easily than do macrophages and therefore are more numerous in exudate or impression smears than in tissue sections. Similarly, cytologic reports describe more neutrophils than do histopathologic reports of granulomas. Fungi, higher bacteria, foreign bodies, and cell debris are predominantly phagocytized by macrophages.

> NOTE: Granulomatous inflammation (i.e., >50% macrophages) suggests the cause was large structures such as foreign bodies, necrotic debris, fungi and certain bacteria that stimulate macrophage proliferation rather than pus formation.

Scanning of the smears is critical to find large, abnormal structures such as burdock awns (golden-brown, linear, barbed plant material) in samples from a lingual foreign-body granuloma. Gross inspection may locate fungal colonies as a spot in one area of a smear, or they may be initially noted as off-white, yellow, or green flecks in a fresh sample. Macrophages are inspected for phagocytized material. In organized hematomas, macrophages contain

phagocytized RBC and RBC breakdown products (e.g., hemosiderin, hematoidin). Macrophages from necrotic lesions contain nuclear fragments and cellular debris. Injection site reactions usually have high numbers of macrophages containing eosinophilic debris (see Color Plate 5F). In steatitis, smears contain oil droplets or rarely yellow fat crystals. The combination of a drop of a fat stain (e.g., Sudan's) and a drop of NMB demonstrates lipid in macrophages. Yeast and hyphae usually stain blue on NMB and Wright's stain; if they stain poorly, their shape may be outlined by background material. To confirm the presence of an unstained organism, one should try another stain. Mycobacteria do not stain and appear as clear slits (i.e., rod-shaped ghosts) in macrophages. Smears may be sent to a referral laboratory for acid-fast staining.

Chronic Inflammation

Chronic inflammatory lesions that lack the abundance of macrophages described in the preceding granulomatous category have a mixture of inflammatory cells including plasma cells, lymphocytes, macrophages, neutrophils, and occasional fibroblasts. This pattern is expected, for example, in canine lick "granulomas," in later stages of healing of an inflammatory lesion (e.g., an old abscess), or in a chronic low-grade inflammatory disease (e.g., proliferative synovitis). Smears from very fibrous lesions are often poorly cellular and might be mistaken for a poor aspirate. Repeat sampling yields similarly acellular samples. Because fibroblasts exfoliate poorly, one should use plasma cells as a cytologic indicator of chronicity. It takes days to weeks for plasma cells to proliferate in an inflammatory lesion.

Eosinophilic Inflammation

Inflammation is usually classified by the most numerous type of WBC. Because eosinophils are normally rare, eosinophils need only exceed 20% to 30% of a significantly large population of WBC to indicate eosinophilic inflammation. Eosinophilic granuloma complex in cats and Siberian husky dogs is diagnosed when smears from a typical lesion in the expected area indicate eosinophilic inflammation with a variable component of macrophages, plasma cells, and mast cells. In cats, eosinophilic plaque and linear granuloma usually have eosinophilic infiltrates and fibroblasts, whereas eosinophilic ulcers may not. Parasites (e.g., *Paragonimus kellicotti*) (see Figure 11-12) and allergic reactions are usually the first rule outs, depending on the area and history (e.g., hypersensitivity reaction to feline rabies inoculation), but neoplastic (e.g., canine mast cell tumor; see Color Plates 6A and 6B) and various infectious, inflammatory diseases, or both may have eosinophilic infiltrates (see the discussion of eosinophilia in Chapter 4).

Lymphocytic Inflammation

A small mass or elevation of a surface may consist of well-differentiated lymphocytes and a variable number of plasma cells. These occur in the nasopharyngeal area, vagina, and intestine, where one might not expect a lymph node or focal lymphoid tissue. This lymphoid hyperplasia may be focal (e.g., focal lymphoid hyperplasia in vagina) or diffuse (e.g., lymphocytic rhinitis). Possible causes of this immune stimulation include viral or mycoplasma infections, but many agents are possible.

Selected Causative Agents

Descriptions of several organisms follow. A cytology or microbiology text or computer program with an atlas is useful (Tvedten, 2000). Granulomatous or pyogranulomatous inflammation suggests larger organisms, but the type of inflammation is not a major differentiating feature of various agents. Diagnosis by microscopic identification is emphasized here. Culture of the organisms and immunologic testing are discussed in Chapter 15. Many organisms have distinct geographic distributions (e.g., salmon disease in Washington, Oregon, and northern California). Anatomic location of the infection (e.g., cryptococcosis in feline nasal cavity) also aids in diagnosis.

The following dimensions are often given in micrometers (μm). A micrometer may be inserted in the microscope's eyepiece. The micrometer's units may be calibrated for each objective with the grid of a hemocytometer. RBC and WBC on the smear may also be used to estimate size. Canine RBC are about 7 μm, and feline RBC are about 6 μm. The approximate diameters of WBC on blood smears are as follows: neutrophils, 14 μm; eosinophils, 16 μm; small lymphocytes, 6 to 10 μm; large

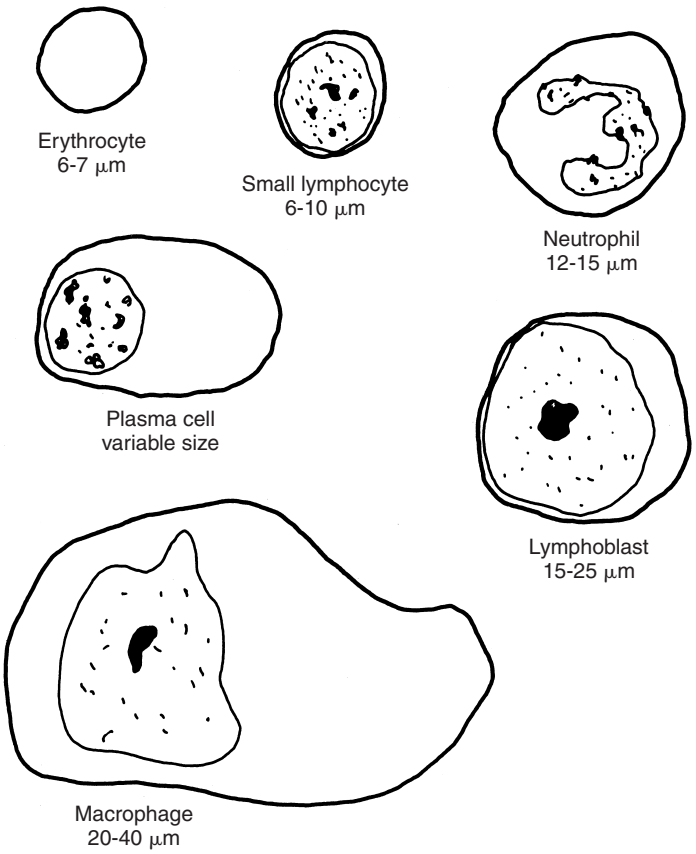

FIGURE 16-5. Cell sizes. Certain cells on smears (especially erythrocytes and neutrophils) may be used as micrometers to judge the size of infectious agents and maturity of unknown cells. These cells are drawn to scale.

lymphocytes, 12 to 15 µm; blast-transformed lymphocytes, 15 to 25 µm; monocytes, 14 to 20 µm; and macrophages, 20 to 40 µm (Figure 16-5). Depending on the thickness of the smear, cells vary in diameter (see Figure 16-2), so these values are only approximate.

NOTE: One should judge the size of organisms by comparing them to the size of erythrocytes or neutrophils.

Fungal Characteristics

Yeast are characterized by the formation of buds on uniformly sized round to oval structures. Spherules such as *Coccidioides*, *Rhinosporidium*, and *Prototheca* form endospores. The organisms are differentiated by size, appearance of buds or endospores, shape, capsule, and location in the body (Figure 16-6). Other structures may mimic yeast, such as fat droplets in urine, especially when two adjacent droplets resemble budding. Unlike yeast, oil droplets are of various sizes and are refractile.

Fungal hyphae have two parallel cell walls and form branches. Hyphae are 3 to 20 µm thick, may have distinct septa, and may form spores or fruiting bodies (i.e., sporophore). Streaks of nuclear debris and lint may mimic hyphae. *Alternaria* is a common contaminant in the air, dust, and smears. It has golden septate hyphae and less frequently diagnostic club-shaped conidia reminiscent of *Microsporum* (see Figure 16-4).

Small pathogenic fungi (e.g., *Histoplasma*, *Sporotrichum*) are in macrophages (see Color Plates 3E and 3F), whereas larger fungi are usually not phagocytized. Contaminant fungi are found anywhere on a slide, including areas away from tissue imprints or fluid smears. If a colony of fungi is found, one should check the stain for fungal contamination by applying the stain to a blank slide.

Histoplasmosis

Histoplasma capsulatum in cats is most consistently found in bone marrow aspirates

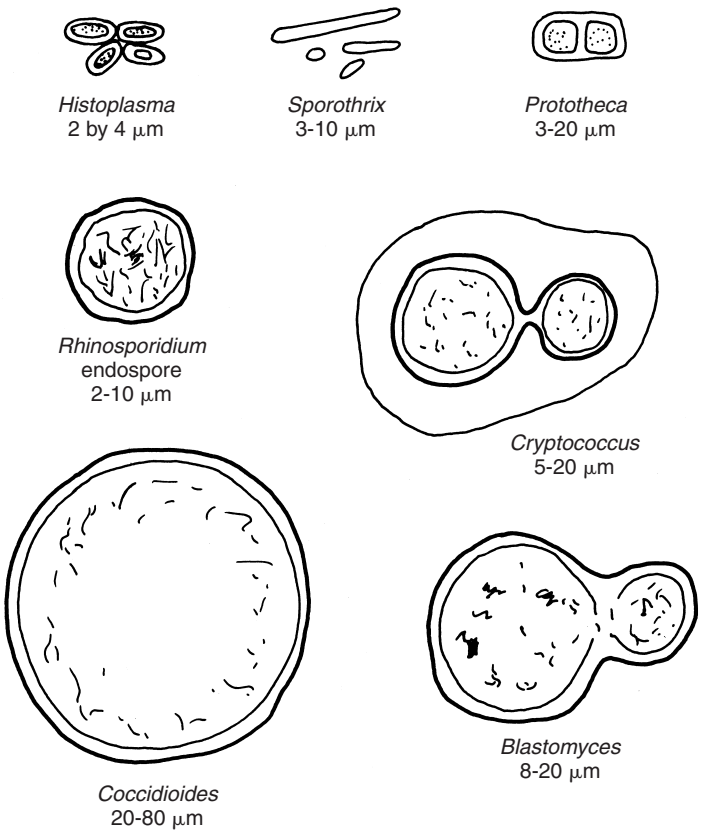

FIGURE 16-6. Comparative morphology of selected yeast and spherical organisms. These are drawn to scale: Size is useful for differentiation.

(Clinkenbeard, Cowell, and Tyler, 1987). Blood smear analysis is an insensitive diagnostic test, but yeast may be found in phagocytes in any body fluid. Buffy coat smears concentrate WBC for examination. *Histoplasma* may be diagnosed by cytology or histopathology of enlarged lymph nodes, liver, or other organs (e.g., colonic scrapings). The small (i.e., 2 to 5 μm) yeasts are in macrophages and occasionally in neutrophils (see Figure 16-6). A phagocyte may contain a few to many yeasts (see Color Plate 3E). Budding may be observed.

Sporotrichosis

Sporothrix schenckii is abundant in samples of draining, ulcerated feline lesions but the yeast is difficult to find in canine lesions. In cats, the yeast is very pleomorphic with round, oval, and fusiform (i.e., cigar shaped) 3 to 10 μm forms (see Figure 16-6) in macrophages, neutrophils, and free in the background (see Color Plate 3F).

WARNING: People acquire sporotrichosis from animals more easily than they do other mycoses.

Cryptococcosis

Cryptococcus neoformans may be found in various tissues. A nasal mass in cats is a classic presentation. This yeast is best identified by the variably thick gelatinous capsule that often doubles the size of the cell (see Figure 11-5 and Figure 16-6). Rare strains of *Cryptococcus* lack a capsule. The yeast itself usually stains clear to eosinophilic. Budding is from a narrow base, in contrast with *Blastomyces*, which has broad-based buds. Cells vary from 8 to 20 μm in diameter. *Rhinosporidium seeberi* occurs rarely in the nose of dogs and produces endospores (2 to 5 μm) that may be confused with cryptococcosis. *Cryptococcus* yeast is well demonstrated on smears stained with Wright's, NMB, or both. India ink preparations are messy and unnecessary. Inflammation may be absent, and the lesion just a glistening mass of yeast.

Rhinosporidiosis

R. seeberi infects the nasal cavity of dogs with the formation of recurrent nonneoplastic polyps. The huge trophic stages (i.e., 60 to 120 µm) and sporangia (i.e., 100 to 300 µm) are diagnostic on histologic sections and occasionally visible on cytologic specimens. The smaller 2 to 10 µm endospores from ruptured sporangia are commonly visible on cytologic preparations (see Color Plate 6F and Figure 16-6). The spherical endospores lack budding and the characteristic capsule associated with *Cryptococcus*.

Coccidioidomycosis

Coccidioides immitis is usually recovered from pulmonary or disseminated lesions in dogs and rarely from cats. It is characterized by large size and internal endospores. Spherical sporangia range from 10 to greater than 100 µm in diameter (see Figure 16-6). Endospores (2 to 5 µm in diameter) are usually in bigger spherules. *Coccidioides* organisms tend to be surrounded by inflammatory phagocytes on smears (Perman, Alsaker, and Riss, 1979). Arthrospores formed in fungal cultures are highly infectious; therefore the microbiologist should be warned if *C. immitis* is possible.

Blastomycosis

Blastomyces dermatitidis is a thick-walled budding yeast (approximately 20 µm in diameter) infecting lungs or other tissues in dogs and occasionally cats. With Wright's stain, the yeast cells are dark blue and are often collapsed and wrinkled from the alcohol dehydration during staining, but they appear more typical with NMB stain. They are best found by scanning the slides. Because the yeasts are large, they are often pushed to the end of the smears (see Figure 11-13 and Figure 16-6).

Candidiasis

Candida albicans is usually a normal flora. It rarely infects a surface (i.e., thrush, moniliasis) or is disseminated. It is a typical thin-walled budding yeast about 2 to 6 µm in diameter. It is differentiated from other yeasts by the formation of pseudohyphae (3 to 4 µm thick), which look like short septate hyphae in cultures and occasionally in tissue samples.

Pityrosporum

Malassezia (*Pityrosporum*) (see Color Plate 3A) is a small budding yeast resembling *Candida*. It is most often found in ear swabs of dogs with chronic otitis that have been nonresponsive to antibiotic therapy. Being able to diagnose chronic yeast otitis alone justifies cytology in private practice. Karen Moriello (Dermatologist, University of Wisconsin) suggests using a metal spatula to harvest yeast from between the toes of dogs that persistently lick their paws or other skin sites. The spatula is designed for transferring small amounts of powder to weighing scales (Fisher brand, Cat. No. 21 401 20) but is effective and not traumatic in skin scrapings. Malassezia in cats may be smaller and resemble a large bacterial coccus.

Protothecosis

Prototheca is a rarely disseminated canine infection. Signs are related to lesions in the skin, eye, and intestine. The round to oval algae are 3 to 20 µm in diameter (usually just smaller than a neutrophil), have a clear cell wall, and may have two or more endospores (see Color Plate 3B and Figure 16-6). Cytologic and histologic diagnosis can be further confirmed by culture and immunofluorescent tests on tissues.

Aspergillosis

Aspergillus spp. is the most common fungus associated with canine nasal infections (see Chapter 11). It is critical to scan many smears to find a fungal colony, because most of the sample is exudate with secondary bacterial sepsis. Finding a septic exudate may suggest a bacterial cause and terminate the search before the primary problem is found. Grossly finding a green *(Aspergillus fumigatus)* or brown *(Aspergillus niger)* mass (i.e., the fungal colony) to use for cytology improves the probability of diagnosis. On scanning, the fungal colony resembles a clump of debris, but on high magnification it is a mass of septate, branching hyphae of comparably uniform thickness (i.e., 3 to 4 µm) (see Color Plate 3D). This is good evidence of aspergillosis. The center of an *Aspergillus* colony may not stain, thus requiring examination of its perimeter. The large (i.e., 300 µm) branching conidiophore with 2.5 to 3 µm spherical conidia is most diagnostic. A cytologic smear occasionally

has only the small, clear, bluish spherical spores that may have a thin, clear halo. See the earlier discussion on hyphae under Fungal Characteristics to differentiate nonpathogenic forms such as *Alternaria*.

Mycetoma

Cutaneous fungal granulomas have a confusing array of identities (e.g., eumycotic mycetomas, maduromycosis, chromoblastomycosis) and are caused by a wide variety of fungi (e.g., *Drechslera, Allescheria, Madurella, Cladosporium, Fonsecaea*). Cytologic diagnosis is limited to identifying a granulomatous or pyogranulomatous inflammation with one or more fungal forms (i.e., hyphae, spherules). Shape, size, and color of fungi on an unstained slide should be recorded. Fungal culture is used for specific causative diagnosis.

Mucormycosis

Mucor and similar relatively nonpathogenic fungi may be found in canine gastric ulcers or other tissues. The hyphae are nonseptate, branching, and wider (i.e., 15 to 20 μm) than those of *Aspergillus* spp. One should confirm by culture.

Alternaria

Alternaria is a ubiquitous contaminant found in various microscopic preparations that must not be confused with truly pathogenic fungi. *Alternaria* organisms have large, golden-brown septate hyphae and characteristic club-shaped conidia with longitudinal and transverse septa (see Figure 16-4). *Alternaria* is rarely pathogenic. Documenting that it has invaded deeper tissues indicates infection and not just surface contamination.

Dermatophytes

Cytology for dermatophytes is covered in Chapter 15. See Color Plate 3C.

Higher Bacteria

Actinomyces, Nocardia, and *Dermatophilus* are higher bacteria. They branch but their thin width of 0.5 to 1.0 μm distinguishes them from thicker fungal hyphae. Fungal hyphae, in contrast, have two distinct cell walls separated by obvious space (see Figure 16-4). *Actinomyces viscosus* is so much more common than *Nocardia* spp. (by bacterial culture) in Michigan that no cytologic attempt is made to differentiate them. *Actinomyces* is characterized by long filaments that occasionally branch and have a beaded appearance on Wright's stain (i.e., variable staining intensity along the filaments). Scanning smears hastens detection of darkly stained bacterial colonies. *Actinomyces* may be coated with an eosinophilic material and form "clubs" radiating out from the colony. Other organisms with the eosinophilic clubbing around colonies include *Staphylococcus* (botryomycosis), *Actinobacillus*, *Coccidioides*, and *Aspergillus*. Colonies in exudate appear grossly as white to yellow granules and should be collected for the smear. *Actinomyces* and *Nocardia* are pleomorphic organisms that may appear as a mixed bacterial infection (see Color Plates 4B and 4C).

Dermatophilus congolensis causes cutaneous infections in dogs less frequently than in other species. One can identify it by soaking skin crusts in saline and then rubbing the underside of the crusts on glass slides. On Wright's stain, characteristic railroad track–like patterns of parallel and longitudinal rows of zoospores (0.5 to 1.0 μm) (Figure 16-7) forming variably thick branching structures are found on squamous cells.

Mycobacteriosis

Mycobacterium lepraemurium, *Mycobacterium fortuitum*, *Mycobacterium bovis*, and some other species cause cutaneous or SC masses in cats and less frequently in dogs (Gross and Connelly, 1983). Feline leprosy (*M. lepraemurium*) lesions occur anywhere on a cat; atypical mycobacteriosis tends to cause fistulous tracts on the ventral abdomen. The organisms are demonstrated by impression smears of the granulomas or smears of the draining tracts stained with acid-fast stains (see Color Plate 4D). Mycobacteria do not stain with Wright's stain, and negatively stained rods appear as clear slits or "ghosts" in macrophages. The smears should be mailed to a cytologist or microbiologist for staining.

One should notify the histopathologist, because finding mycobacteria requires special processing. Organisms tend to localize in clear lipid droplets in the center of granulomas, and they are lost during alcohol and xylene steps if routine slide preparation is performed. Frozen sections retain the organisms and their staining ability. Culture is required to differentiate feline leprosy from

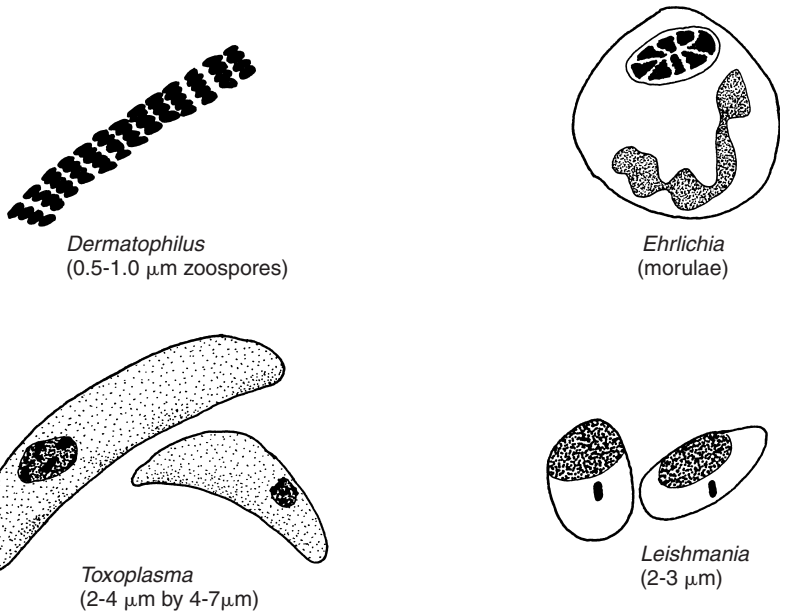

FIGURE 16-7. Four organisms identified by cytology. *Dermatophilus* is often on squames. An *Ehrlichia* morula is a cluster of bodies in the cytoplasm of white blood cells (WBCs). *Toxoplasma* and *Leishmania* are protozoa. *Leishmania* have a rod-shaped kinetoplast.

atypical mycobacteriosis. One should advise the microbiologist to use media for atypical mycobacteria. Cytology, histopathology, and culture may need to be repeated.

> NOTE: The pathologist should be advised of the possibility of *Mycobacterium* infection, because special processing of histopathologic samples improves the chance of finding the organisms.

Salmon Disease

Neorickettsia helminthoeca may be identified in macrophages from lymph nodes of infected dogs. A moderate to large number of coccoid to rod-shaped bodies (0.3 μm in diameter) are spread through the cytoplasm of macrophages and may form morulae. Macchiavello's stain is excellent for rickettsiae, but Wright's-type stains are suitable. Trematode eggs are in a dog's feces 1 week after infested fish are eaten.

Ehrlichiosis

Finding an *Ehrlichia canis* morula (i.e., raspberry-like cluster of tiny bodies) in circulating WBC, cytologic smears of lung, or synovial fluid is diagnostic but is rare. See *E. canis* diagnosis discussion in Chapters 5 and 15. *Ehrlichia ewingii* infection is often associated with acute disease and polyarthritis. Morula are often visible in peripheral blood and synovial fluid neutrophils (see Figure 16-7). A Swedish strain of canine ehrlichiosis commonly has morulae in neutrophils.

Toxoplasmosis

Toxoplasma gondii is rarely found in macrophages and neural, ocular, or muscle tissue. The actively dividing forms (i.e., tachyzoites) are crescent shaped (i.e., 2 to 4 μm × 4 to 7 μm), with a nucleus at one end (see Figure 16-7). The shape may not be discerned when several are packed in a cell, but it becomes apparent when freed from a ruptured cell. Smaller bradyzoites in stable tissue cysts remain infective for a long time. Similar protozoa to consider are *Sarcocystis* and *Leishmania*. Active *Toxoplasma* infection is best diagnosed serologically (see Chapter 15). Small (i.e., 10 to 12 μm) coccidial oocysts of *Toxoplasma* are briefly (i.e., 2 weeks) shed in feces by cats after ingestion of infected meat. This suggests the enteric infection but is too transient for consistent diagnosis. Other coccidial oocysts are usually larger (i.e., 20 to 40 μm).

Leishmania

Leishmania may be found in macrophages in bone marrow, lymph nodes, or splenic aspirates of dogs with visceral infections (see Figure 16-7). One should note the rod-shaped kinetoplast in the protozoa. Infected dogs often come from countries around the

Mediterranean (e.g., Greece, Spain). PCR testing is specific and sensitive (see Chapter 15).

Cytauxzoonosis

Cytauxzoon felis is a tick-transmitted, highly fatal protozoal disease of domestic cats. Cytauxzoonosis is diagnosed antemortem by finding the small signet ring intraerythrocytic stage (i.e., >50% of cases) or finding large macrophages containing schizonts of developing *Cytauxzoon* organisms in lung, liver, spleen, lymph node, or bone marrow aspirates or impression smears.

Proliferative Masses (Neoplasia)

Initial Decisions

The two major initial conclusions are (1) the smears represent the mass, and (2) the lesion is a noninflammatory proliferation of tissue cells (e.g., benign neoplasm, malignant neoplasm, focal hyperplasia, normal tissue). These conclusions must be made before considering tissue type or cytologic malignancy of the mass. Finding a moderate to large number of cells of one type should be representative of the mass.

For mammary neoplasm cytology, more than 100 cells per slide were considered adequate for evaluation (Allen, Prasse, and Mahaffey, 1986). When a small number of tissue cells are present, the risk is increased that they do not represent the mass. This is especially so when the sample is diluted with blood, exudate, or other fluid.

Cells indicate a proliferative tissue mass if they are noninflammatory tissue cells of one type (i.e., a monomorphic population). This conclusion is often made difficult by concurrent inflammation and necrosis. The higher the percentage of inflammatory cells in the population, the lower is one's confidence that the mass is not primarily inflammatory.

Cell Typing

Shape of the cells, association with other cells (especially in tissue fragments), and cytoplasmic features are used to indicate the tissue of origin. One should not expect to routinely identify exact cell type by cytology; rather, one should determine whether it is an epithelial, a mesenchymal (i.e., connective tissue, spindle cell), or a round cell tumor. Histopathology should be used for more specific classification of neoplasms. Additional information such as location (e.g., mammary tumor) usually suggests a specific diagnosis. Some very malignant neoplasms, for example, may lack differentiating features to identify the cell type, but the cytologic conclusion of "very malignant neoplasm—type undetermined" is sufficient. A mass may be composed of multiple tissue types (e.g., an epithelial neoplasm has variable to abundant connective tissue stroma). Melanoma is a neoplasm that may have an epithelial- or connective-tissue appearance (or both).

Epithelial cells are best indicated by distinct, tight, cell-to-cell junctions (Figure 16-8). Epithelial cells form surfaces with tight cell junctions that persist to a variable degree on smears. One should be cautious of interpreting adjacent, crowded cells on a thick part of the smear as having epithelial junctions where the cells simply flatten along edges of contact. Instead, one should look for distinct linear junctions, complete with formation of angles and corners. Epithelial cells may form layers, so an epithelial cell may be identified in a pocket formed by an adjacent epithelial cell (the way one hand fits in the palm of another). Tissue fragments in smears may retain acinar structure with lumina (glandular origin) or papillary structures (i.e., fingerlike epithelial projections with a central core of connective tissue stroma). Tissue architecture is best shown with semitransparent stains like NMB. Well-differentiated epithelial cells may retain squamous, polyhedral, cuboidal, or columnar shapes and cilia. Secretory material such as mucus, granules, or vacuoles suggests glandular epithelium.

Some other tissues such as mesothelium (and synovium) form surfaces and may mimic epithelial tissue. This plus the anaplastic appearance of reactive mesothelial cells may easily cause a misdiagnosis of carcinoma in pleural, pericardial, and peritoneal fluids. Often cytologists are sent fluid smears with large anaplastic cells for differentiation of reactive mesothelial cells from neoplastic cells. Because no one can consistently determine the difference between these cell types, clinicians should not expect "experts" to do so. Certain neoplasms like lymphoma can be diagnosed consistently in fluids. Mesothelial cells often form epithelial-type papillae, which are mesothelial cell–lined, fingerlike projections formed during irritation of a surface (e.g., villous proliferation in *Actinomyces* pleuritis or hemopericardium). Synovial cells,

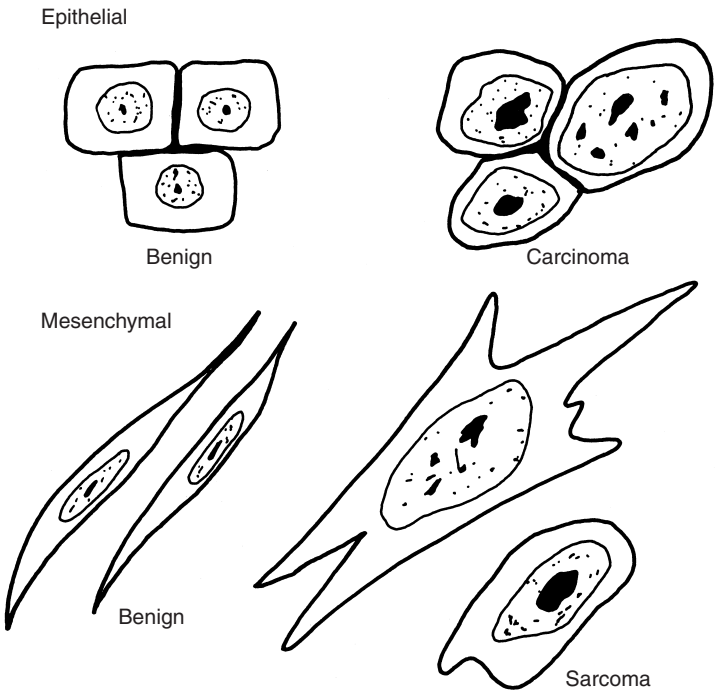

FIGURE 16-8. Benign and malignant examples of epithelial and mesenchymal cells. The reader should note the tight intercellular junctions of the two clusters of three epithelial cells *(top)*. The two pairs of individualized cells *(bottom)* have the elongated spindle or stellate shapes of mesenchymal cells. Malignant nuclei *(on the right)* are larger, more variable, and have more irregular nucleoli and chromatin.

endothelial cells, and melanocytes may also mimic epithelial cells.

NOTE: No one can consistently differentiate reactive mesothelial cells in peritoneal, pleural, or pericardial fluid from neoplastic cells (e.g., carcinoma, mesothelioma). It is a waste of time and money to send fluid smears with this type of large anaplastic cells to a cytologist for differentiation.

Cells are classified as mesenchymal (e.g., fibrous, osseous, muscle, or neural connective tissue) mainly by an elongated shape forming tail-like or conic extensions (see Figure 16-8). Two tails are found on spindle-shaped cells (see Color Plate 6D). Three or more tails are on stellate cells. Nuclei tend to be more oval than with epithelial cells. Many mesenchymal cells on smears may appear rounded, so one should look for a tendency of the cells to have spindle or stellate forms. Mesenchymal cells tend to lack sharply defined cell borders. Occasionally a matrix produced by the cells (e.g., pink osteoid, collagen strands) is found around or among cells. Cytoplasmic structures suggest the specific cell type. Melanocytes usually have a fine golden-brown to black pigment. Melanin granules may be rod shaped in melanocytes or round and larger in melanophages. Hemosiderin may be mistaken for melanin but is usually larger and may be mixed with green or yellow pigment that is more easily recognized as hemosiderin or accompanies erythrophagocytosis. Osseous cells sometimes have prominent pink cytoplasmic granules. Columnar epithelial cells may mimic spindle cells, because the point where the base of the cell pulled away from its attachment to the basement membrane often pulls out to a thin, pointed tail. The other end of the columnar cell has a flat surface with cilia to indicate its true type.

Four tumors classically placed in the round cell category are (1) lymphoma and other hematopoietic cell neoplasms, (2) mast cell tumor, (3) transmissible venereal tumor, and (4) canine histiocytoma. Characteristics of these are illustrated in Figure 16-9 and described later. The round cell category should also include poorly differentiated tumors with large round cells. Malignant large round cell neoplasm is a descriptive cytologic

NOTE: One should not limit the differential diagnosis of round cell neoplasms to the four classic types, because anaplastic melanoma, plasmacytoma, and anaplastic carcinoma may cytologically have discrete, only round cells on cytologic smears.

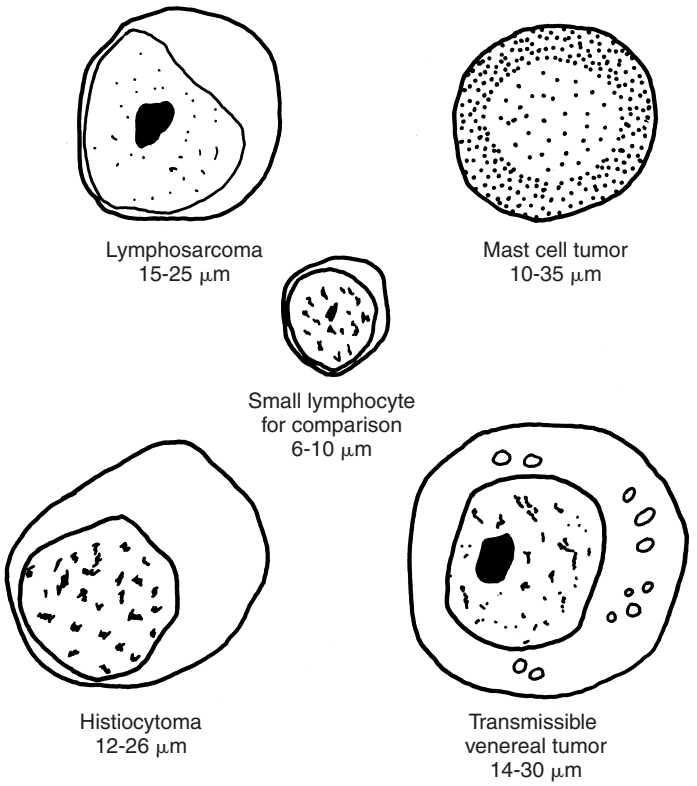

FIGURE 16-9. Round cell tumors. Cell characteristics of the four classic round cell tumors are illustrated with a small lymphocyte for size comparison.

diagnosis sufficient for a working diagnosis in these cases.

Malignant Criteria

After deciding that the cytologic population represents a proliferative mass, one evaluates malignant criteria. One should record the malignant criteria of the smear and quantitate the amount (i.e., small, moderate, great). A complete description with the relative abundance of evidence leads more easily to a diagnosis.

How are malignant criteria converted into a conclusion? Grading systems attempt to convert subjective observations into quantitative measures. A scoring system for malignancy of canine mammary neoplasm has been critically evaluated (Allen, Prasse, and Mahaffey, 1986). It allowed objective consideration of how conclusions were made. More critical evaluations of veterinary cytology, which is often more art than science, are needed. One point was added for each of 10 cytologic criteria that had been shown to correlate significantly with histopathologic conclusions of malignancy. A score of 0 to 3 was benign, 4 to 7 was inconclusive, and 8 to 10 was malignant. It is apparent that several malignant criteria are required, and many samples have inconclusive amounts of evidence. Therefore one should not expect a diagnosis from every sample. This system had few false-malignant diagnoses (i.e., high specificity), but the sensitivity in identifying malignancy was only 17% to 25%. When the cutoff for a test is set high for best specificity (i.e., few false-malignant diagnoses), the sensitivity is usually lower (i.e., more false-benign diagnoses). High specificity is preferred to sensitivity, especially if euthanasia may be determined by the diagnosis.

One should not expect to count malignant criteria until a "magic" number is reached. Even with rigid definitions of each malignant criterion, the mammary neoplasm scoring system has a wide inconclusive range. The number of malignant criteria noted in each case varies, because cytologists vary. A nucleus that appears large to one observer may seem normal to another. People vary in thoroughness. Use of a numbering system is weakened if equal weight is given when a few cells barely have the change, compared with when most cells have great alterations. No single feature or group of features always proves malignancy, and the conclusion is a subjective impression.

Malignancy is best indicated by nuclear variability (see Color Plates 6B and 6C). Variation in nuclear size (i.e., anisokaryosis) and very large nuclear size are obvious even on poor quality smears. Stronger evidence of malignancy is indicated by increasing nuclear size and anisokaryosis. Anaplastic cells tend to have large nuclei and minimal cytoplasm, resulting in a high nuclear:cytoplasmic (N:C) ratio. Bizarre nuclear shape variation, such as with pseudopods and marked convolutions, is strong evidence. Multinucleation in cells not normally multinucleated occurs in malignant and reactive cells. Molding of a nucleus around the nucleus of an adjacent cell is evidence that the cells grew next to each other rather than a strong criterion of malignancy.

Variation in intranuclear structures is better evaluated with a stain such as NMB, Sano's trichrome, or Pap's stain than the Wright's-type stains that give indistinct nuclear detail. Variable-appearing chromatin is strong evidence of malignancy but is hard to describe. It is recognized with experience as "looking malignant." Malignant chromatin patterns are characterized by variability in size, shape, and distribution of chromatin granules. Irregular parachromatin clearing appears as an area of clear space separating the chromatin granules unevenly. The variability gives a coarse, irregular chromatin pattern. Well-differentiated cells tend to have uniform chromatin with even distribution. Active nuclei have fine chromatin (i.e., small granules) and increased clear space between chromatin (i.e., parachromatin area). This makes the nucleus appear lighter than a smaller, well-differentiated nucleus with condensed chromatin (i.e., larger, denser granules). Active nuclei occur in malignant and nonmalignant cells.

Malignant cells often have prominent nucleolar variation, such as excessive variation in the number of nucleoli (i.e., varying from one to five or more), large nucleolar size (may be larger than an RBC), variation in nucleolar shape (i.e., jagged, sharply pointed, irregular nucleoli), and variation in size of nucleoli even within the same nucleus. Nucleolar variability is strong evidence of malignancy and easily recognized on NMB-stained smears.

Malignant nuclei lack uniformity among themselves. Benign cells have consistent numbers (e.g., 1 to 3) of small- to moderate-sized round, smooth or indistinct nucleoli. A few mitotic figures are often given excessive weight as malignant criteria. Mitotic figures are routinely found in nonneoplastic macrophages in cytospin smears of exudates. Mitotic figures do indicate malignancy if they are numerous or abnormal. Abnormal mitotic shapes include excessive numbers of chromosomes (e.g., tripolar metaphase plate instead of the normal two rows of chromosomes) and lag chromatin (i.e., a chromosome separated from the other chromosomes in the mitotic figure).

Cytoplasmic changes are weak indicators of malignancy. A basophilic cytoplasm indicates active protein synthesis and abundant RNA. A large nucleolus also indicates active RNA synthesis and thus protein synthesis. Protein synthesis is often high in malignant cells but also in active nonneoplastic cells (e.g., hyperplasia, reactive change). Normal hepatocytes, for example, have large nucleoli reflecting active production of m-RNA and protein synthesis. Malignancy of a mast cell tumor is often indicated by variability in the number, size, and distribution of cytoplasmic granules. Abundance of cytoplasm and differentiated features (e.g., retaining a columnar shape or cilia) are features of benign proliferations.

Degenerative changes mimic malignant changes. Nuclei and nucleoli swell with cell damage (see Figure 16-2), and the larger nuclear size, light nuclear color, and nucleolar prominence make the damaged cell appear malignant. *One should not evaluate cells with broken cytoplasmic boundaries or with partial leakage of nuclear material out of the nucleus!* Even apparently intact cells may be swollen and degenerating. The best indication of cell death is chromatin that has lost its granular, stippled appearance on Wright's stain and appears streaked or smudged. The degeneration is less obvious on NMB-stained cells, so it is easier to mistake the degeneration for malignancy.

> NOTE: Degenerative changes in cells from necrotic areas, old samples, or during cell processing mimic malignant changes. Debris may also resemble infectious agents.

Cells degenerate quickly in certain fluids like unbuffered saline and urine. Cells collected directly from bronchial brushings, for example, have much better cell detail than do cells collected with a saline flush. Cells degenerate within minutes in regular saline, which is acidic (i.e., pH 6) and lacks inorganic ions and glucose for cellular metabolism. Hanks' balanced salt solution (HBSS) preserves cell

morphology and is a better fluid to retrieve cells when cellular detail is critical (e.g., neoplastic diagnosis). The cost of HBSS prevents its routine use in veterinary medicine. Mediocre cellular detail is adequate for evaluation of exudates and sepsis but not for diagnosis of neoplasms. To prevent excessive cell degeneration, one should make direct smears as quickly as possible and avoid prolonged cell storage in fluids. Adding protein such as 5% to 10% bovine serum albumin solution or several drops of serum or plasma to fluid before centrifugation (e.g., cytocentrifuge) tends to minimize cell degeneration.

Selected Cytologic Diagnoses

Mammary Neoplasms

Cytologic evaluation of mammary masses is used to plan surgical removal. Lack of cytologic evidence of malignancy permits local resection and removal for histologic diagnosis. Cytologic evidence of malignancy or the gross presentation of the neoplasm may indicate more radical surgery. Draining lymph nodes should be checked cytologically for metastasis by cytology. Even mildly altered epithelial cells in lymph node smears give confident proof of malignancy and metastasis, whereas great changes in epithelial cells from mammary tumors are more equivocal.

Ten cytologic criteria were significantly correlated to histopathologic conclusion of malignancy (Allen, Prasse, and Mahaffey, 1986). Cytologic evaluation was performed on modified Sano's trichrome stain of smears fixed in 95% ethanol while still wet. The malignant criteria were (1) variable nuclear size, (2) giant nuclei (more than twice normal), (3) distortion of nuclear or cytoplasmic membranes, (4) high N:C ratio (i.e., >1:2), (5) irregular chromatin shapes, (6) variable chromatin size, (7) parachromatin clearing (i.e., a discrete pale area in a more dense chromatin pattern), (8) variable nucleolar number (i.e., >3), (9) abnormal nucleolar shape, and (10) macronucleoli (more than two times normal). Abnormal nucleolar shape was defined as "not round or oval." Nuclear membrane distortion was indentation of nuclear shape by a cytoplasmic organelle, such as a vacuole, in contrast with nuclear molding, which was the molding of a nucleus about another cell's nucleus without cell crowding.

Abnormal mitotic figures (e.g., asymmetry, trisomy) always denoted malignancy but were rare. Similarly, cytoplasmic projections indicated malignancy but were infrequent. Neither nuclear molding, irregularly thick nuclear margins, cellularity of the smear, poor intercellular cohesion, nor multinucleation allowed differentiation between benign and malignant neoplasms. Fine-needle aspirates of mammary tumors were more cellular and had better tissue architecture than impression smears, scrapings, or nipple secretions. Spindle cells on smears did not differentiate simple from complex or mixed tumors. In another survey, only 8 of 19 histologically confirmed mammary carcinomas were identified as such by fine-needle cytology (Griffiths, Lumsden, and Valli, 1984).

> NOTE: Mammary tumor cytology should be used for tentative diagnoses, with histopathology required for definitive diagnosis.

Perianal Gland Tumor

Cells of perianal gland tumor (i.e., hepatoid tumor) resemble hepatocytes with a square or polyhedral shape and abundant cytoplasm (see Color Plate 6E). The cytoplasm has characteristic granularity with Wright's stain. Round nuclei have one or two prominent nucleoli. Clusters of cells may retain the typical long columnar pattern visible in tissues. The site of the mass aids in identification. Metastasis is rare (i.e., to the lung), but cellular appearance remains distinctive. Cytologic appearance may not reflect biologic malignancy, because benign-appearing cells may be found at metastatic sites.

> NOTE: Malignancy of perianal gland neoplasms is best determined by histologic evidence of vascular invasion. Cytology consistently identifies the tumor as perianal gland (i.e., hepatoid) but may not identify the degree of malignancy.

Transitional Cell Carcinoma

Cytologically, transitional cell carcinoma is diagnosed best by fine-needle aspirates of identifiable masses, thickenings, or both in the bladder. Diagnosis by finding significant numbers of well-preserved, malignant-appearing epithelial cells in urine is less consistent. Cells in urine rapidly degenerate and swell, causing nuclear and nucleolar enlargement mimicking neoplasia and making diagnosis difficult.

Malignant cells in urine usually indicate transitional cell carcinoma, but prostatic carcinoma or others are possible. Prostatic neoplasms have more columnar to cuboidal cells.

Lipoma

Diagnosis is rapid and simple for a common tumor. Lipoma diagnosis by cytology should be routinely done by veterinarians and does not require a cytologist. Smears grossly have clear droplets of lipid that do not dry. Staining with Sudan's or another fat stain is unnecessary. The Wright's-stained smears may lack cells or have a variable number of adipocytes singly or in tissue fragments. Adipocytes are large cells with a small dark nucleus on one edge. The thin cell membrane may appear wrinkled as cells shrink when alcohol in Wright's stain removes intracellular lipid. Mature fat has the same appearance as a lipoma. Lipomas may be traumatized, hemorrhagic, fibrotic, or inflamed. These changes are indicated by macrophages, other inflammatory cells, blood, and fibroblasts.

Mast Cell Neoplasms

Mast cell neoplasms are confidently diagnosed by the finding of a moderate to large population of mast cells with a variable number of eosinophils from a mass. The round, distinctly granular cells of more differentiated tumors are easily identified on Wright's stain (see Figure 16-9 and Color Plate 6A). Some modified Wright's stains may fail to stain mast cell granules. All mast cell tumors should be regarded as potentially malignant. Although anaplastic, poorly granular mast cells clearly indicate malignancy, some cytologically well-differentiated mast cell tumors have metastatic potential. Histologic grading of canine cutaneous mast cell tumors has been associated with differences in survival. Classification was from grade 1 (i.e., well-differentiated, round monomorphic cells with no mitotic activity and round nuclei with condensed chromatin) to grade 2 (i.e., intermediate) to grade 3 (i.e., pleomorphic cells with irregularly shaped cells, vesiculated nuclei with one or more prominent nucleoli, binucleation, frequent mitotic figures, and cytoplasmic granules that were indistinct, fine, or not obvious).

Feline cutaneous mast cell tumors without splenic involvement are usually benign, and survival in a small survey of 14 cats did not correlate with a grading system similar to the canine system (Buerger and Scott, 1987). In histiocytic-type cutaneous mast cell tumors in young Siamese cats, granules may be difficult to find (see also the discussion of mastocytemia in Chapter 4).

Cytologically, cutaneous mast cell tumors are subclassified by degree of differentiation. Some nuclear criteria of malignancy may be hard to identify on Wright's-stained cytologic smears, because the nuclei may be obscured by granules and often stain poorly owing to the mast cell's heparin content. NMB is better for nuclear detail. Cytoplasmic characteristics of malignancy in dogs include variation in the size, density, and prominence of the granules. Malignant cells have fewer and finer granules (see Color Plate 6B). Granules may polarize to one end of malignant cells. Anaplastic cells are larger (i.e., 12 to 35 μm instead of normal 10 to 20 μm), with mitotic figures and binucleation (Duncan and Prasse, 1979).

Histiocytoma

A histiocytoma can be a troublesome cytologic diagnosis, because cells are less distinctive than other round cell tumors. Cells may exfoliate poorly, and secondary inflammation may occur. The cells are 12 to 26 μm and round to oval with distinct cell boundaries. They occasionally have indented nuclei without apparent nucleoli (Duncan and Prasse, 1979). Chromatin is fine, and mitotic activity is minimal. Cytoplasm on Wright's stain is pale blue. The N:C ratio varies and is usually 1:1. Human pathologists unfamiliar with this benign tumor often consider it malignant. Lymphomas may have a histiocytic appearance and be confused with a histiocytoma, so caution is advised if the presentation is not typical for histiocytoma (e.g., a small dome-shaped skin mass in a young dog). Cutaneous canine plasmacytoma is a round cell tumor of older dogs.

Transmissible Venereal Tumor

Transmissible venereal tumor is a round cell tumor with discrete cells that may show moderate to marked variation in cell size. The round cells are 14 to 30 μm in diameter. Round to oval nuclei have a prominent nucleolus and linear, cordlike chromatin. The cytoplasm is a pale hyaline blue with distinct vacuoles (Duncan and Prasse, 1979). Mitotic activity is common. Location of the mass on the body (i.e., genital area, mouth),

plus being from an endemic area, supports the diagnosis.

Epidermal Inclusion Cyst

Epidermal inclusion cysts are common skin lumps in dogs. Contents of the cyst mainly consist of mature stratified squamous epithelial cells, so the diagnosis is made by finding large numbers of squames (very mature, nonnucleated squamous cells) and keratinized debris on smears. (Note: Occasional squames are common contaminants on smears, mainly from fingerprints.) Other debris, including cholesterol crystals, may be present. Traumatized cysts may be inflamed or hemorrhagic.

> NOTE: An epidermal inclusion cyst is a common skin mass. Cytologic diagnosis is easily made by finding large numbers of anuclear squames and often cholesterol crystals.

Hematoma and Seroma

Hematomas are fluctuant masses containing fluid with a variable amount of blood in variable states of degeneration. Recently formed hematomas have aspiration smears resembling a blood smear. Platelets, if present, suggest active bleeding, because platelets are rapidly lost from blood outside blood vessels. With time, RBC are ingested (see Color Plate 5C) and converted to various blood pigments by infiltrating macrophages. Granular or crystalline pigments have various colors (i.e., blue, green, black, gold, yellow). The proteinaceous fluid from a seroma has fewer RBCs.

Hepatic Cytology

Hepatocytes normally have abundant granular cytoplasm and a round nucleus with a prominent nucleolus. Binucleation (i.e., diploid) is normal in a minority of hepatocytes. Increased numbers of binucleated hepatocytes suggests hyperplasia. Common alterations include vacuolar degeneration (e.g., fatty liver, glucocorticoid hepatopathy), inflammation (e.g., chronic hepatitis), cholestasis, and neoplasia. Aspiration cytology may identify these processes, although histopathology better identifies and quantitates the disorders. When well-outlined, variably sized hepatocytic vacuoles are visible, fatty change is expected and can be proven by applying one to two drops of Sudan's stain and counterstaining with one to two drops of NMB (see Color Plate 5E). Canine glucocorticoid hepatopathy has accumulation of glycogen and not fat. Glycogen accumulation appears as light pink, cytoplasmic clearing instead of distinct vacuoles. Cholestasis is indicated by swollen, green bile canaliculi between hepatocytes or by blue-green granular pigment within hepatocytes.

Inflammation may be difficult to prove cytologically, because inflammatory infiltrates are often minimal in amount in hepatitis or cholangiohepatitis. If only a few lymphocytes or neutrophils are on a hemodiluted smear, it is hard to tell whether WBCs are from peripheral blood or a mild inflammatory infiltrate (e.g., cholangiohepatitis). The capillary collection (not aspiration) technique described earlier is recommended to harvest hepatocytes with minimal hemodilution, so the presence of WBCs better reflects a hepatitis. Because plasma cells and vacuolated macrophages are not found in peripheral blood (and do not contaminate a sample by hemodilution), they are more consistent indicators of inflammation than are neutrophils and lymphocytes. Extramedullary hematopoiesis is indicated by nucleated RBC and megakaryocytes. Neoplasia is diagnosed by previously described criteria. Metastatic neoplasia is easier to recognize cytologically than a hepatoma.

Lymph Node Cytology and Lymphoma

Aspiration cytology is the preferred test to evaluate enlarged lymph nodes. Cytology may provide evidence of metastasis and other disorders even in normal sized nodes. Because lymphoid cells are fragile and large lymph nodes are often necrotic, many smears may not be diagnostic if inadequate numbers of intact cells are present. One should scan the smears for diagnostic areas with adequate numbers of intact cells. Then a differential cell count should be performed in a thin area with good cell morphologic detail, while other areas are examined to ensure that the differential count is representative. One uses the population's composition to reach one or more of the conclusions in Table 16-1.

Lymphoid cells in a normal lymph node are about 5% to 10% lymphoblasts and 75% to 90% small- to medium-sized, well-differentiated lymphocytes. Plasma cells are infrequent (i.e., 0% to 3%) except in some commonly reactive nodes (e.g., mesenteric, submandibular). Criteria used to differentiate lymphoblasts from well-differentiated, small to medium lymphocytes vary. Lymphoblasts are as large or

TABLE 16-1. Cytologic Lymph Node Conclusions

CONCLUSION	CYTOLOGIC FEATURES
Normal	Normal lymphoid cell population, no lymph node enlargement
Hyperplastic	Normal cell population, enlarged lymph node
Reactive	Similar to hyperplastic but includes increases in plasma cells, lymphoblasts, granulocytes, or macrophages with hemosiderin or debris
Lymphadenitis	Large population of one or more nonlymphoid leukocytes; organisms may be present
Metastatic neoplasia	Population of nonlymphoid cells with cytologic evidence of malignancy, excessive numbers, or atypical location
Lymphoid neoplasia	Usually a predominant population of immature lymphoid cells

larger than neutrophils, have a light, fine nuclear chromatin pattern, and usually have one or more nucleoli. In contrast, the small lymphocyte is just larger than a RBC but smaller than a neutrophil. The lymphocyte nucleus has coarser and darker chromatin and no visible nucleolus (see Figure 16-5). Medium-sized lymphocytes are grouped with small lymphocytes as well-differentiated cells if they have a mature, coarse chromatin pattern and no visible nucleolus. *Prolymphocyte* was an old hematologic term for immature lymphoid cells with large nuclear size and immature chromatin patterns; they are included with "lymphoblasts" in this paragraph even though their nucleoli may not be visible (see later discussion on updated Kiel classification of lymphomas).

Lymph node cytology is routinely used for diagnosis of lymphoma (i.e., lymphosarcoma [LSA]) (see Chapter 4). The percentage of lymphoblasts or other obviously immature forms is the major diagnostic criterion in lymphoma. (This chapter mainly uses a simple system where lymphoid cells are divided into lymphocytes, plasma cells, and lymphoblasts. See also the following discussion.) Lymphoblasts greater than 50% of cells on the smear allow a diagnosis of lymphoma. Confidence in a diagnosis of lymphoma increases with the percentage of lymphoblasts. Fewer than 50% lymphoblasts may be found in more uncommon forms of lymphoma. Atypical morphology, other than immaturity, is not a consistent or sensitive indicator of lymphoma. When present, features such as bizarre nuclear and cytoplasmic shapes (e.g., "hand mirror cells" with cytoplasmic pseudopods), numerous mitotic figures, or irregular chromatin patterns add to one's confidence in a diagnosis of lymphoma.

The reader should note the term "lymphoblast" in the previous description refers to a large, immature lymphoid cell and does not correlate to the updated Kiel classification of lymphoblastic lymphoma (Raskin and Meyer, 2001). In the updated Kiel classification, lymphoblasts do not have a prominent nucleolus, so there can be confusion concerning to what the term *lymphoblast* refers. Immunoblasts and centroblasts have one or more prominent nucleoli and are what older, simpler hematologists and cytologists have called and continue to call *lymphoblasts*. Immunoblastic, centroblastic and lymphoblastic lymphomas have large immature lymphoid cells called lymphoblasts in the simplified classification of this chapter.

Frequent necrosis in lymph nodes with lymphoma can cause aspiration of loose necrotic debris. A thick background of excessive cell debris on aspirate and impression smears interferes with staining. One may find only a small percentage of intact and well-stained cells around the thin perimeter of the smear. Lysed cells (i.e., "naked" nuclei) and partially lysed normal cells have swelling of the nucleus and nucleolus. This creates a larger, lighter-stained nucleus with a prominent nucleolus resembling a lymphoblast. Failure to ignore these damaged cells leads to an erroneously high percentage of lymphoblasts and perhaps an incorrect conclusion of lymphoma. One should not look at damaged cells. Repeated aspiration may be required to obtain a diagnostic sample.

Several forms of lymphoma exist that have small to medium lymphoid cells that lack prominent nucleoli and that are more difficult to diagnose cytologically. Some examples are pleomorphic small T-cell lymphoma, mycosis fungoides, pleomorphic medium to large T-cell lymphoma, and chronic lymphocytic leukemia (Raskin and Meyer, 2001). Immunophenotyping aids in classification and prognosis of lymphomas.

Chronic lymphocytic leukemia (CLL) is not diagnosed by immaturity of the population, because the neoplastic lymphoid cells have mature chromatin patterns. CLL is not so uncommon and should be considered as a cause of lymphadenopathy if a cytologically *monotonous* lymphoid population of medium-sized lymphocytes is present. The lymphocytes have moderate to abundant cytoplasm, so they are larger than normal lymphocytes. CLL may be diagnosed by histopathology showing architectural replacement of normal structure of affected lymph nodes, spleen, and bone marrow. Usually a prominent lymphocytosis is found in the leukogram (see Chapter 4).

Highly reactive lymph nodes may have an increased percentage (e.g., 10% to 25%) of lymphoblasts and some very large lymphoblasts that might suggest lymphoma. Lymphoblasts accounting for much less than 50% of the cell population and the heterogeneity of the population with variation in size and maturity of lymphoid cells and abundant plasma cells, however, are useful indicators of a reactive and hyperplastic node compared with the usually predominant population of lymphoblasts in lymphoma. An admixture of small to medium lymphocytes with the lymphoblasts and increased numbers of plasma cells supports a more conservative diagnosis of a reactive lymph node.

Reactive and hyperplastic lymph nodes are essentially the same: a benign, expected response to some irritant in its drainage field. A hyperplastic lymph node has a normal lymphoid population but is larger in size, owing to an immunologic stimulus (e.g., Demodex in the skin). If the stimulus is associated with hemorrhage or inflammation so that the node has hemosiderin-laden macrophages, increased neutrophils and eosinophils, or an increased number of lymphoblasts, the diagnosis tends to be reactive lymph node.

If greater than 5% to 10% of the cells from the lymph node are mature, nonlymphoid WBCs, the diagnosis is lymphadenitis. The predominant type of WBC determines the type of inflammation (i.e., neutrophilic, granulomatous, pyogranulomatous, eosinophilic). The likely causes were discussed previously. If the majority of the cells from the lymph node are nonlymphoid WBCs, nodular abscessation or leukemic infiltration is the diagnosis based on maturity of the WBCs. A pattern seen with chronic granulocytic leukemia is when the lymph node aspirate looks like bone marrow with mixed granulopoiesis.

Metastatic neoplasia is diagnosed if adequate numbers of malignant-appearing nonlymphoid cells are present. A few large anaplastic cells can occur in nonneoplastic reactive nodes; unless cells are very distinctive (e.g., forming definite epithelial patterns), a confident diagnosis requires many cells (e.g., 50 to 100). Occasional mast cells are normal in lymph nodes and can undergo hyperplasia. Hence many mast cells must be found to diagnose mast cell neoplasia. Macrophages with hemosiderin mimic melanocytes, so one should be careful diagnosing metastatic melanoma. Inflamed lymph nodes may be fibrotic; therefore one must accept a few fibroblasts without diagnosing sarcoma. A few cells prove metastasis if they are foreign to the lymph node, such as stratified squamous epithelial cells, perianal gland cells, and mucin-secreting epithelial cells.

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17

Gary Osweiler

Laboratory Diagnostic Toxicology

PRINCIPLES OF TOXICOLOGIC DIAGNOSIS

Clinical observations may help determine the organ systems affected. Tables 17-1 through 17-6 show probable toxicoses associated with different organ systems and clinical signs (e.g., seizures, hepatic failure, vomiting), but very few toxicoses have pathognomonic signs. In addition, many signs caused by poisoning (e.g., vomiting, seizures) are also caused by infectious, metabolic, and endocrine diseases. A thorough history and physical examination should be completed to determine access of the animal to potential toxicants. Telephone instructions should include having the owners collect any potential baits, vomitus, or other suspect materials for possible chemical analysis. Important historical findings include changes in location, changes in food source, recent chemical applications (e.g., spraying for insects, fertilizing a lawn, changing radiator coolant), whether the animal is free to roam, and distance to public or commercial areas.

SUPPORTING CLINICAL LABORATORY TESTS

After clinical data is obtained from history and physical examination, laboratory testing is used to identify and characterize pathophysiologic effects typical of specific toxicants (e.g., basophilic stippling and nucleated red blood cells [nRBCs] without reticulocytosis suggest lead toxicosis; high serum osmolality and an increased anion gap are consistent with ethylene glycol [EG] toxicosis). Table 17-7 summarizes clinical laboratory tests that may be altered by specific toxicoses. A major use of the toxicology laboratory is to determine presence and possibly amount of a specific toxicant. For some toxicants (e.g., strychnine) any amount is diagnostic of a toxicosis; other toxicants depend on quantitation (e.g., lead).

Blood/plasma is the major vehicle for body transport of toxicants. Whole blood should be collected in anticoagulant (ethylenediaminetetraacetic acid [EDTA] or heparin unless otherwise specified). Vomitus or feces

TABLE 17-1. Common Toxicants of the Nervous System

TOXICANT	COMMON SOURCES	COMMENTS
Signs of excitation		
Aminopyridine	Bird control baits.	Tremors, ataxia, may be hyperexcitable.
Caffeine, other methylxanthine alkaloids	Chocolate, coffee, tea; stimulant or antidrowsiness pills sold OTC.	Excitement, exaggerated reflexes, hyperreflexia; cardiac arrhythmia, hyperpnea, vomiting. Alkaloids detectable in GI contents, blood, or urine.
Cyanide	Cyanogenic plants (apple, peach, cherry seeds); fumigants; rodenticides (rarely); industrial chemicals.	Excitement, agitation, seizures progressing to ataxia, weakness, and collapse; also salivation, hyperpnea, pink mucous membranes. Very rapid course. Test stomach contents, blood for cyanide, or liver and muscle from dead animals.
Lead	Paint (pre-1970) or specialty paints, lead objects (drapery weights, fishing sinkers).	Intermittent vomiting and behavioral changes; tremors, ataxia, seizures, altered vision, anorexia.
Metaldehyde	Snail and slug baits; solid fuels sold OTC.	Continuous tremors, occasional seizures; incoordination and cerebellar ataxia; nystagmus, salivation also prominent.
Pyrethrins, pyrethroids	Active ingredients in insecticides for pets and homes.	Tremors, hyperexcitability, excitement, occasional seizures; sometimes alternate with depression.
Strychnine	Mole and gopher baits, usually less than 0.05% for below ground use.	Acute onset of hyperesthesia, hyperreflexia progressing to tetanic seizures exacerbated by external stimuli; rapid shallow respiration, tachycardia.
Tremorgenic mycotoxins	Penitrem A on moldy walnuts, other nut products; less commonly, spoiled dairy products.	Tremors, ataxia, and hypermetria are characteristic and may be continuous; exacerbated by exercise or stress. Test stomach contents or suspect materials for penitrem A or other tremorgens. Available laboratory testing is limited.
Zinc phosphide	Used as alternative to anticoagulants; sold OTC in some states. Bait has mild garlic or acetylene odor.	Excitement, stimulation and seizures may include tremors and "running fits." Alternative sign may be depression. Vomiting, colic often accompany the neurologic signs.
Signs of depression or coma		
Alcohols	Beverages, disinfectants, brewery residues.	Depression, disorientation early, followed by coma, respiratory depression, acidosis, cardiac arrest. Alcohol residues appear in blood and urine.
Barbiturates	Access to tablets or capsules; occasionally in meat from animals euthanitized with barbiturates.	Depression, loss of reflexes, hypothermia, hypotension, coma, respiratory failure. Barbiturates in blood or urine of living animals; liver or kidney of dead animals.
Bromethalin	Relatively new rodenticide, often used as an alternative to anticoagulant rodenticides.	Signs may include posterior ataxia and weakness, sometimes vomiting resulting from cerebral edema. Available laboratory testing is limited.
Citrus oils	Citrus extracts used in insecticides and repellents (e.g., D-limonene). Most hazardous to cats.	Depression, ataxia, coma, and pronounced hypothermia. Citrus extracts are conjugated in the liver and excreted in urine. Few laboratories offer routine testing for these products.
Carbon monoxide	See Table 17-5.	See Table 17-5.
Ethylene glycol	Automobile radiator antifreeze solutions. Also used to prevent freezing in water pipes of vacant houses or mobile homes.	Profound depression early is due to alcohol-like inebriation; later signs of depression are due to severe metabolic acidosis.
Hydrocarbons (aliphatic and aromatic)	Petroleum distillates and pine oil compounds; most available in paint thinners, mineral spirits, cleaning solutions; exposed by spilling on skin, footpads, or by inhalation of fumes.	Generally there is initial depression and ataxia similar to ethanol intoxication. High dosage leads to vomiting, possible liver and/or kidney damage, severe depression, coma, respiratory depression, and death.

Continued

TABLE 17–1. Common Toxicants of the Nervous System—cont'd

TOXICANT	COMMON SOURCES	COMMENTS
Signs of parasympathetic stimulation		
Blue-green algae	Blue-green algae (*Anabaena* spp. and *Microsystis* spp.) are most prevalent in lakes and farm ponds during late summer.	In addition to sudden death (neuromuscular paralysis) and hepatotoxicity, blue-green algae may inhibit cholinesterase, causing signs of salivation, vomiting, tremors, and dyspnea. Water samples should be submitted fresh and fixed (addition of 1 part formalin/9 parts water).
Nicotine	Tobacco products, specialty insecticides (e.g., nicotine sulfate). Also used occasionally in capture guns.	Initial signs are due to early depolarization (tremors, excitement, lacrimation, vomiting), followed by paresis, ataxia, and complete collapse with death from respiratory paralysis. Nicotine alkaloid is readily detected in stomach contents, blood, or urine.
Signs of parasympatholytic agents		
Atropine	Plant, *Atropa belladonna,* may be grown as ornamental in gardens.	Causes dry mucous membranes, mydriasis, tachypnea, tachycardia, hyperthermia, disorientation, visual dysfunction, GI stasis. Readily detected in blood and/or urine.
Scopolamine	*Hyocyamus niger* and *Datura* spp. are common plant sources. Also available as pharmaceutical.	Signs and effects are similar to those with atropine (above).
Ataxia, incoordination, weakness, or paralysis		
Aminoglycoside antibiotics	Kanamycin, neomycin, streptomycin, gentamicin	Effects are due to postsynaptic receptor blockade of neuromuscular junctions, results in paresis, paralysis, and death from respiratory failure. Testing of blood and/or urine may establish exposure.
Botulism	*Clostridium botulinum* growth in decaying organic matter, especially with high protein content.	Lower motor neuron paralysis results in muscle weakness, difficult deglutition, progressive paresis and paralysis, mydriasis, dysphagia. Suspect material for stomach contents may be cultured or injected in mice as a bioassay, followed by protection tests with antitoxin.
Cholinesterase inhibitors	See *Signs of parasympathetic stimulation.*	Test for confirmation of cholinesterase inhibitors as described above.
Modification of behavior		
Atropine/ scopolamine	See *Signs of parasympatholytic agents.*	Behavioral effects of disorientation and possible loss of vision appear secondary to overdose of atropine. Diagnosis of atropine overdose is described above (*Signs of parasympatholytic agents).*
Ethanol	See *Signs of excitation.*	See *Signs of depression or coma.*
Lead	See *Signs of depression or coma.*	Lead exposure may cause changes in mental abilities, lost recognition of familiar persons, belligerence, and hysteria.
Lysergic acid (LSD)	Sources are some plant forms (Morning glory seeds, ergotized grains) or illicit street drugs.	May be profound behavioral changes ranging from excitement and hallucinations to deep depression. Analysis of vomitus or stools may help to establish exposure.
Marijuana	Illicit street drugs, prepared from leaves or seeds of *Cannabis sativa*. Sometimes prepared in foods such as brownies.	Behavioral changes may include depression, excitement, hallucinations with barking at unknown stimuli; nystagmus, vomiting, and diarrhea. Diagnosis by recognition of suspect material and/or testing of blood or urine for *cannabis* alkaloids.

OTC = over-the-counter; GI = gastrointestinal.

TABLE 17-2. Toxicants of the Digestive System

TOXICANT	COMMON SOURCES	COMMENTS
Direct irritants		
Acids, alkalis, aldehydes	Batteries, cleaners and bleaches, disinfectants	Salivation, dysphagia, acute glossopharyngeal swelling, cough, nasal discharge. Laboratory testing of suspect materials may establish source, but animal testing for these substances is usually not practical or helpful.
Petroleum distillates	Solvents, paint thinners, furniture cleaners, gasoline, kerosene	Coughing, vomiting, choking. Possible sequelae are aspiration pneumonia and central nervous system depression. Agents are difficult to detect in blood or tissues by laboratory tests.
Volatile oils	Turpentine, gum spirits, pine oil, eucalyptus oil, pennyroyal oil, lemon oil	Acute gastroenteritis with vomiting and possibly diarrhea. Systemic effects can include seizures, delirium, depression, and coma.
Hyperemic/necrotic gastroenteritis		
Amanita phalloides (Death cap, Death angel)	Wild, toxic mushroom. Most prominent in eastern or western seacoast regions.	Acute clinical signs are hemorrhagic gastroenteritis with vomiting and bloody diarrhea, hepatosis after a latent period of 12-24 hours. Available laboratory testing is limited.
Arsenic, antimony, bismuth	Older insecticides or herbicides, including ant baits; old paint pigment. Most uses of arsenic are restricted or canceled. Antimony may be present in caustic pastes.	Acute vomiting, followed by moderate to severe diarrhea changing from watery to necrotizing and hemorrhagic within 24-48 hours. Hypotension, shock and renal tubular damage are additional effects. Analysis of urine or gastrointestinal contents can help to establish exposure. Liver and kidney concentrations from postmortem specimens can be helpful.
Iron	Accidental access to dietary iron supplements.	Acute gastroenteritis, shock, vascular collapse and death, liver damage 1-2 days later. Test for liver function, assay total serum iron and total iron-binding capacity.
Staphylococcus toxins	Spoiled foods, especially egg and high-protein products left at room temperature.	Severe vomiting and diarrhea may develop after short latent period (<3 hrs). Cultures may identify the potential bacterium but alone are not confirmatory.
Gastroenteritis/nonhemorrhagic		
Digitalis glycosides	Accidental access to prescription medicines; plant sources including foxglove, oleander.	Cardiac signs may be preceded or accompanied by colic and vomiting.
Lead	See Table 17-1.	Vomiting is intermittent.

TABLE 17-3. Hepatotoxins

TOXICANT	COMMON SOURCES	COMMENTS
Acetaminophen	Over-the-counter pain killer and anti-inflammatory drug.	Initial signs of vomiting, cyanosis and facial edema are followed by icterus, depression and mild methemoglobinemia.
Amanita phalloides	Wild mushroom, most common in east or west coast areas.	Acute vomiting is followed in 1-2 days by liver failure.
Blue-green algae	Algal forms, commonly concentrated by low water and prevailing winds.	Acute gastroenteritis, vomiting, and hemorrhagic diarrhea. Algae containing water fixed in formalin 1:10 can be examined for presence of blue-green algae. Frozen samples are used for toxic activity by mouse bioassay.
Iron	Dietary supplement pills.	See Table 17-2.
Petroleum distillates	Solvent in paints, paint thinners, paint strippers; fuels including gasoline, kerosene.	Liver damage may accompany initial signs of neurologic dysfunction.

TABLE 17-4. Cardiovascular Toxicants

TOXICANT	COMMON SOURCES	COMMENTS
Cardioactive glycosides	Digoxin and related prescription drugs; foxglove, oleander, *Bufo* spp. toads.	Vomiting, colic, diarrhea, followed by weakness, bradycardia, and arrhythmia.
Cholecalciferol	Vitamin supplements, rodenticides. High dosages cause signs in 12-36 hours.	Hypercalcemia, azotemia, bradycardia, arrhythmias, dystrophic calcification.
Cocaine	Illegal street drug.	Tachycardia and arrhythmias. Test plasma or urine.
Ionophores	Poultry coccidiostats; ruminant feed supplements. Dangerous to dogs.	Trembling, weakness, stiffness, recumbency, cardiovascular insufficiency. Analysis of stomach contents; cardiac muscle biopsy for diagnosis.
Yew bushes	Ornamental evergreen shrubs with strap-like, two ranked, dark-green leaves.	Sudden death several hours after ingestion; nervousness, trembling, bradycardia and arrhythmia. Test for yew alkaloids in stomach.

may provide evidence of recent oral exposure, biliary excretion, or both. Many organic toxicants are excreted in urine. Hair may document chronic accumulation of a toxicant (e.g., arsenic), showing prior exposure even when gastrointestinal and organ concentrations are no longer detectable. Baits and other environmental samples may establish a source of poison or probable route of exposure. Environmental samples include food, water, suspected toxic plants, baits, pesticides, household products, medications, and solvents. Empty containers may also be useful.

When animals die the clinician should perform a thorough necropsy and collect appropriate specimens to examine for lesions and analyze for toxicants. Stomach contents may contain plants, foreign objects, abnormal colors (e.g., marker dyes associated with pesticides), tablets, or capsules. Specimens of appropriate organs and tissues should be (1) saved fresh by freezing and (2) placed in 10% neutral buffered formalin for microscopic examination. Specimens should include brain, liver, kidney, cardiac muscle, stomach and intestines, lung, urine, feces, and any other tissues appearing abnormal. Many toxicoses can be presumptively diagnosed by a characteristic lesion or lesions (e.g., coumarin anticoagulants cause coagulopathy, usually with hemothorax; EG toxicosis is routinely diagnosed at postmortem by microscopic examination of renal impression smears or routine H&E histopathology).

OBTAINING AND SHIPPING SPECIMENS

Samples must be free from contamination (e.g., environmental agents, medications, preservatives) and sealed individually in glass or plastic containers. Although standard evacuated blood or serum tubes are adequate for most body fluids, some trace elements (e.g., zinc) require special trace element tubes (i.e., Vacutainer, royal-blue cap). The clinician should never use preservatives unless laboratory instructions specifically recommend them. Serum should be separated from the clot before shipping. Unless otherwise indicated, freezing of samples for toxicologic analyses is the best means of preservation. For unusual analyses, or if in doubt about specimens needed, the clinician should consult a toxicologist or call the laboratory for information.

Applicable U.S. Postal Service rules regarding biologic, infectious, and toxic materials should be followed. This generally means protective bagging around the entire submission and use of United Nations–approved packaging materials (usually indicated in catalogs for packaging materials). If sample integrity and chain of custody are important (e.g., insurance claims, litigation), the clinician should seal the shipping box, place a copy of the transmittal letter in an envelope marked "Invoice," stick the envelope to the outside of the sealed box and, if possible, ship it directly to a specific receiving person who has been notified in advance.

SELECTING AN ANALYTICAL LABORATORY

Because most analytic toxicology testing is performed in a referral laboratory, the laboratory should be selected based on assurances of quality and service. Important characteristics of a good laboratory include active application of quality-control programs, accreditation with recognized certifying agencies, modern

TABLE 17-5. Toxicants of the Blood and Bone Marrow

TOXICANT	COMMON SOURCES	COMMENTS
Hemolysis		
Onions, garlic	Accidental access to large amounts in prepared foods or by direct ingestion.	Acute signs are typical of hemolytic crisis.
Phenothiazine anthelmintics	Older, little-used anthelmintic.	Acute hemolytic crisis may occur. Phenothiazine in urine becomes oxidized to a red color. Phenothiazine can be detected in blood or urine, but routine testing is not offered by many laboratories.
Zinc	Zinc metal objects, galvanized food or water containers, zinc ointments, pennies minted after 1983.	Vomiting and diarrhea in combination with pale mucous membranes and icterus.
Methemoglobin		
Acetaminophen	OTC analgesic and anti-inflammatory. See Table 17-3.	Hepatotoxicosis is prominent. Blood may display dark-brown color due to methemoglobinemia.
Aniline dyes	Shoe polish, inks, paints. More prominent in older products.	See Nitrites, below.
Chlorates	Soil/contact herbicide or soil sterilant. Used where prolonged suppression of plant growth is desired. Accidental ingestion of granules may occur.	See Nitrites, below.
Nitrites	Lawn fertilizers, explosives, meat-curing agents, contaminated well water.	Methemoglobinemia, cyanosis, weakness, depression, hyperpnea. See Acetaminophen in text for analysis of methemoglobin.
Carboxyhemoglobin		
Carbon monoxide	Poorly vented heaters, automobile exhaust.	Depression, weakness, somnolence, coma, and death. Mucous membranes are bright pink and blood is cherry-red to pink.
Aplastic anemia and thrombocytopenia		
Benzene	Gasoline and industrial or commercial products as a solvent. Small animals could be at risk by inhalation exposure.	Causes initial central nervous system signs of tremors, ataxia. Cardiac fibrillation may occur at high acute exposures. Prolonged exposures usually cause pancytopenia. Serum iron is increased and fetal hemoglobin may be increased. Anemia is macrocytic with relatively few reticulocytes that are generally immature.
Estrogens	Used to correct mismating, treat prostatic hyperplasia, induce abortion, or reduce urinary incontinence.	Lethargy, weakness, pale mucous membranes, petechial hemorrhages, hematuria, melena. Pancytopenia, reduced reticulocyte count.
Coagulopathy		
Anticoagulant rodenticides	OTC baits for rodent control in homes and business.	Dermal and mucosal petechiae and ecchymoses, hemoptysis, epistaxis, melena, pale mucous membranes, weakness, dyspnea, subcutaneous hematoma, muffled heart and lung sounds, depression, and death.

OTC = over-the-counter.

analytical equipment, and availability of a laboratory veterinarian for consultation and interpretation.

DIAGNOSIS OF SPECIFIC TOXICANTS BY LABORATORY ANALYSIS

Chemical analysis is the final and most important confirmatory procedure for toxicoses. Specific selected common toxicants are addressed in more detail later. In addition, a differential list of toxins is provided for each organ system, along with common sources for each toxicant and comments about distinguishing clinical or laboratory features of the toxicant (see Tables 17-1 to 17-6).

Acetaminophen

Indications • Intoxication with acetaminophen (ACM) is likely in any cat with a history

TABLE 17-6. Common Toxicants Affecting the Kidneys

TOXICANTS	COMMON SOURCES	COMMENTS
Aminoglycoside antibiotics	Primarily kanamycin, neomycin, and gentamicin	Usually occurs with extended treatment. Initial findings are polydipsia/polyuria followed by vomiting and azotemia.
Ethylene glycol	Automobile radiator antifreeze fluids	Initial signs are in central nervous system with ataxia and depression, followed by metabolic acidosis, oxalate nephrosis, and azotemia.
Fungal toxins	Ochratoxin or citrinin in refrigerated table foods (cream cheese, nuts)	Signs are polydipsia, polyuria from renal tubular nephrosis.
Halogenated hydrocarbons	Solvents in commercial products, paints, cleaning agents	Initial signs may include inebriation, incoordination, and depression followed in 1-3 days by toxic tubular nephrosis. Clinical laboratory findings reflect acute tubular injury.
Metals	Many metals and metalloids are potent tubular toxicants.	Effects and signs are typical of tubular nephrosis. Samples for analysis of metals should include blood, urine, kidney, and liver.
Nonsteroidal anti-inflammatory agents	Ibuprofen, naproxen	Renal damage and clinical laboratory findings are similar to those for the aminoglycoside antibiotics. See Table 17-1.
Plants (non-oxalate)	Easter lily (*Lillium* spp.), daylily (*Hemerocallis* spp.)	Cats appear most at risk to these plants. The lilies have a potent tubular toxicant. Suspect animals should be tested for renal function. No toxicant analyses currently available.
Vitamin D_3	Vitamin supplements and vitamin D_3 (cholecalciferol)-based rodenticides	Signs after latent period of 18-24 hours; progress over 1-3 days to polyuria, polydipsia, renal pain on palpation.

TABLE 17-7. Clinical Laboratory Tests That May Be Altered by Toxicoses

LABORATORY PARAMETER	TOXICANT(S)	EFFECT ON VALUES
Alanine amino transferase	Acetaminophen Benzimidazole anthelmintics Halogenated hydrocarbons (e.g., halothane, chloroform)	Moderate to massive increase depending on dosage.
Ammonia	Ammonium-based fertilizers Toxic liver injury	Increased
Anemia, nonregenerative	Lead, cadmium, zinc Cancer chemotherapy Phenylbutazone Chloramphenicol Estrogen therapy	Varies
Anemia, regenerative	Acetaminophen Copper Onions	Heinz bodies indicate oxidant injury from toxicants listed.
Basophilic stippling	Lead	Accompanied by nucleated red cells.
Bile acids	Acetaminophen Aflatoxins Diethylcarbamazine Corticosteroids Thiacetarsamide	Early onset of mild to moderate increase, often preceding other parameters of liver injury.
Bilirubin	Acetaminophen Aflatoxins Thiacetarsamide	Increased
Blood pH	Aspirin Ethylene glycol Methanol	Acidosis

Continued

TABLE 17-7. Clinical Laboratory Tests That May Be Altered by Toxicoses—cont'd

LABORATORY PARAMETER	TOXICANT(S)	EFFECT ON VALUES
Calcium, serum	Cholecalciferol rodenticides	Increased
	Ethylene glycol; oxalates from rhubarb	Decreased
	Phosphate enemas	
Casts, renal	Aminoglycoside antibiotics	Renal epithelial damage as sequel to renal tubule toxicosis.
	Arsenic (cats)	
	Cadmium	
	Lilies (most species)	
	Nonsteroidal anti-inflammatory drugs	
Chloride, serum	Ammonium chloride	Mechanisms of elevation include loss of concentrating ability, laboratory interference.
	Amphotericin	
	Bromides (laboratory error)	
	Lithium	
Coagulation factors	Coumarin based rodenticides (e.g., warfarin, brodifacoum, bromadialone)	Prolonged PT and APTT associated with loss of factors II, VII, IX, X.
Creatine kinase	Ionophores (e.g., monensin, lasalocid, salinomycin)	Increased
Crystalluria	Oxalate-containing plants (e.g., rhubarb, oxalis)	Oxalate crystals in urine sediment
	Ethylene glycol metabolites	
Gamma glutamyl transpeptidase	Glucocorticoids	Moderate increase
	Barbiturates	
Glucose	Sodium monofluoroacetate (compound 1080)	Increased due to blockage of TCA cycle.
Hemoglobinuria	Acetaminophen	Elevated, mild to severe.
	Chlorate herbicides	
	Copper	
	Onions	
	Propylene glycol (cats)	
	Snake venom	
	Zinc, metallic	
Leukocyte count	Benzene	Neutropenia with likely left shift.
	Chloramphenicol	Chemical neutropenia is generally due to inhibition or destruction of stem cells.
	Estrogens (dog)	
	Phenylbutazone	
	Thiacetarsemide	
Magnesium, serum	Gentamicin	Decreased, secondary to nephrosis or hypercalcemia.
	Vitamin D-induced hypercalcemia	
Methemoglobin	Acetaminophen	Increased more than 40% MetHb causes clinical signs.
	Benzocaine	
	Chlorate herbicides	
	Copper	
	Nitrites	
Osmolarity	Aspirin	Hyperosmolarity and high osmolar gap
	Ethanol	
	Ethylene glycol	
	Phosphate enema	
Porphyrinuria	Lead, hexachlorobenzene	Moderate increase
Pancytopenia	Benzene derivatives	
	Chemotherapeutics	
	Thallium	
Phosphate, serum	Cholecalciferol rodenticides	Increased due to vitamin D
	Cestrum diurnum (jessamine)	
Potassium, serum	Digitalis glycosides	Increased
	Nonsteroidal anti-inflammatory drugs	
Sodium, serum	Excess salt intake	Increased
	Low water intake	
Thrombocyte count	Cephalosporins	Decreased
	Estrogens	

APTT = activated partial thromboplastin time; PT = prothrombin time.

of ACM administration (especially if dyspnea or cyanosis is present). One 325 mg tablet may be toxic to cats.

Sample Collection • Plasma (from EDTA or heparin-preserved blood) or serum and urine are the preferred antemortem samples. Blood concentrations usually peak 4 to 6 hours after ingestion. Samples should be refrigerated if analyzed within 24 hours or frozen if longer delays are expected. A blood smear should be examined for Heinz bodies. Antemortem whole blood can be tested for methemoglobin. Methemoglobin is unstable and must be tested within 4 hours or stabilized by hemolysis of the whole blood with an equal amount of sterile water. The clinician should contact the laboratory in advance for recommendations for handling and submitting samples for methemoglobin analysis. Toxic concentrations in tissues have not been established.

Analysis • ACM is measured in blood or plasma. If the test is available, blood glutathione values are depressed. Moderate (i.e., 10% to 50%) to marked (i.e., >50%) numbers of *large* Heinz bodies are expected in intoxicated cats. In normal cats up to 10% of RBCs may contain *small* Heinz bodies (see Chapter 3). Hemoglobinuria or hematuria may be present; methemoglobin concentration is increased.

Normal Values • Data for animals are scarce, but ACM values less than 100 μg/ml are not likely toxic.

Danger Values • Not established in cats; human ACM values greater than 300 μg/ml are indicative of hepatopathy.

Artifacts • Other agents producing methemoglobinemia or hemoglobinuria (e.g., aniline dyes, copper salts, nitrites, onions; see Table 17-5) can mimic ACM's effects.

Causes • ACM toxicosis is most common in cats; dogs are uncommonly poisoned. Exposure is usually due to persons not familiar with ACM toxicosis to cats. Other causes of Heinz bodies and methemoglobinemia should be eliminated (see Table 17-5 and Chapter 3). Evidence of methemoglobinemia, moderate to marked numbers of large Heinz bodies, or both accompanied by positive test results for ACM or its metabolites supports a diagnosis of ACM toxicosis.

Alkaloids

Indications • Intoxications with alkaloids should be considered in patients with central nervous system (CNS) excitation, including seizures (see Table 17-1), with or without vomiting, and possible exposure to alkaloids.

Sample Collection • After absorption, alkaloids may be concentrated in the liver and eventually excreted in urine. Plasma values may be low compared with urine values, unless death occurred very rapidly before excretion in urine (i.e., <1 hour). Preferred samples are vomitus or urine from live animals and any suspect baits (e.g., bird toxicants [4-aminopyridine]). Blood or plasma may contain significant levels of some alkaloids (e.g., the methylxanthines [caffeine, theobromine]). Some alkaloids (e.g., strychnine) are difficult to detect in antemortem blood. Postmortem samples should include liver, kidney, and urine. Refrigeration is adequate for preservation of alkaloids.

Analysis • Alkaloidal compounds of toxicologic importance to small animals include 4-aminopyridine, amphetamines, atropine, caffeine and other methylxanthines, cocaine, and strychnine. Screening analyses may be performed by thin-layer chromatography (TLC). Quantitation of TLC results can be performed by ultraviolet (UV) or visible spectrophotometry or by high-performance liquid chromatography.

Normal Values • For the alkaloids listed previously, normal values are not expected; their presence in the body indicates exposure to an exogenous source. For some (e.g., strychnine), any amount supports a diagnosis of toxicosis.

Danger Values • Analysis confirms exposure, but toxic concentrations are not well established for most common alkaloids.

Artifacts • Many plant compounds and drugs contain nontoxic heterocyclic nitrogen compounds that react as alkaloids. Simple spot tests depending on colorimetric reactions measuring only a general alkaloidal reaction can incorrectly implicate toxicosis because of a false-positive test result. A minimum of TLC after appropriate solvent extraction is recommended and followed, if necessary, by an alternative confirmatory instrumental procedure.

Causes • Poisoning with alkaloids needs to be differentiated from other poisons (see Tables 17-1 and 17-2), other causes of CNS excitation, and other causes of vomiting (see Chapter 9). Diagnosis is usually confirmed by a combination of history and clinical signs in conjunction with demonstration of the alkaloid in stomach contents or body fluids.

Amitraz

Indications • Intoxication with amitraz should be considered in patients with vomiting, depression, ataxia, frequent defecation, or diarrhea, as well as in animals with intentional use or misuse or accidental exposure to amitraz via dips, tick collars, or some livestock sprays. Advanced systemic signs can include bradycardia, hypotension, and convulsions.

Sample Collection • Blood is not a good sample (low amitraz concentrations), but urinary metabolites may allow diagnosis. Amitraz on hair or in vomitus can be analyzed when exposure is uncertain. Samples of vomitus, liver, kidney, skin, hair, brain, or lungs can be used in postmortem cases.

Analysis • Gas liquid chromatography using a nitrogen-phosphorus detector can be used to quantitate amitraz.

Normal Values • Not expected in normal tissues.

Danger Values • Not established.

Causes • Usual causes of amitraz intoxication are substantial therapeutic overdose or inadvertent exposure of a species (e.g., cats) for which amitraz is not recommended. Other toxins (see Table 17-2) and other causes of vomiting and diarrhea need to be eliminated (see Chapter 9). Clinical signs can appear similar to those of organophosphate (OP) or carbamate toxicosis; these may need to be ruled out by appropriate testing (e.g., acetylcholinesterase [AChE]).

Anticoagulant Rodenticides

Indications • Hemorrhage, prolonged activated coagulation time (ACT), prolonged prothrombin time (PT) or prolonged activated partial thromboplastin time (APTT) (or both), or a combination thereof suggests intoxication with anticoagulant rodenticides (see Table 17-5).

Sample Collection • Whole blood (in EDTA or heparin), serum, or urine may be used for analysis of coumarin-based rodenticides. The clinician should use a small-gauge needle for blood collection to minimize hemorrhage from the venipuncture site. Stomach contents or vomitus may contain little or no anticoagulant, because clinical signs occur after a 1- to 2-day latent period. Postmortem samples should include liver and kidney.

Analysis • Anticoagulant rodenticides of toxicologic importance to small animals include warfarin, pindone, chlorophacinone (first-generation) and bromadiolone, diphacenone, and brodifacoum (second generation). Analytic techniques can range from initial screening with thin-layer chromatography to quantitation with high performance liquid chromatography, UV spectroscopy, or fluorescence detection using suspect baits, blood, serum, or urine from poisoned animals. Baits commonly contain 0.05% active ingredient. Blood concentration may be very low (ppb range) when highly potent rodenticides are used (e.g., bromadiolone, diphacenone). Liver and kidney from poisoned animals may contain both parent compound and hydroxylated metabolites.

Hydroxylated metabolites are excreted in the urine and may be present for 1 to 2 days after exposure ceases or for much longer (several weeks) if second-generation anticoagulants are involved. Brodifacoum residues may persist in tissues for up to 120 days (Lipton and Klass, 1984). Indirect evidence of vitamin K antagonism can be gained by clinical laboratory testing for nonfunctional vitamin K–dependent coagulation factors (PIVKA), but confirmatory diagnosis is most certain when the rodenticide or its metabolites are detected.

Normal Values • Anticoagulant rodenticides are not expected in blood or tissues; values less than 1 ng/ml are not associated with clinical signs of toxicosis.

Danger Values • Serum or plasma concentrations vary widely depending on initial dose and duration of exposure and may range from 1 to 10 ng/ml during toxicosis by brodifacoum (DuVall et al, 1989). The liver appears to concentrate brodifacoum as much as 1000-fold over plasma or serum. Diphacenone and

bromadiolone concentrations at necropsy may be less than 1 ppm in blood or liver of clinically poisoned dogs.

Causes • In a patient with a bleeding problem, toxicity must be differentiated from other causes of factor deficiencies, as well as thrombocytopenia and disseminated intravascular coagulation (see Table 17-5 and Chapter 5). Significant concentrations of anticoagulant rodenticides in conjunction with elevated ACT, PT, and APTT with or without PIVKA confirms a diagnosis. Because most current rodenticides use the second-generation group, the clinician should assume that residues are present in urine for several weeks.

Aspirin

Indications • Intoxication with aspirin should be considered in patients with vomiting, fever, and laboratory findings consistent with metabolic acidosis (see Chapter 6). Cats are at much greater risk than dogs. History of recent administration of aspirin to cats, especially if dose is excessive (i.e., >75 mg/kg for more than 7 days), suggests aspirin toxicosis.

Sample Collection • EDTA blood is analyzed for thrombocytopenia and Heinz bodies and plasma is analyzed for serum salicylate concentration. For tests performed within 24 hours, refrigeration is adequate preservation. Plasma held longer than 24 hours for salicylate analysis should be frozen. Salicylate metabolites may be present in liver and kidney, but toxic concentrations are not established.

Analysis • A qualitative presumptive urine test involves adding a few drops of 10% ferric chloride to 1 ml of urine. In the presence of salicylates, a purple color will form. High-performance liquid chromatography allows more definitive and quantitative testing of urine or plasma.

Normal Values • Normal values are not well established in animals; expected human therapeutic plasma concentrations are less than 50 mg/dl.

Danger Values • Serum or plasma salicylate concentrations indicate exposure, but toxic thresholds are not well established in animals. Human toxic blood concentration is 50 to 100 mg/dl; fatal intoxication in a cat has been associated with a blood concentration of 60 mg/dl.

Causes • Most aspirin toxicosis occurs in cats. A presumptive diagnosis is established in a cat with a history of aspirin administration and vomiting. Diagnosis can be confirmed by plasma salicylate determination.

Ethylene Glycol

Indications • Intoxication with ethylene glycol (EG) should be considered in patients with known or suspected ingestion of EG. Ataxia, depression, and vomiting are consistent with EG intoxication; seizures may occur. Cats are more susceptible than dogs on a body weight basis.

Sample Collection • Samples collected in whole blood (in EDTA or heparin), serum or plasma, and urine are required initially. Electrolytes, acid-base status, osmolality, and anion gap should be determined promptly (see Chapter 6). Blood samples taken within 6 hours of ingestion are most likely to be positive for EG. After that, acid intermediates are expected (e.g., glycolic acid). Kidney should be obtained at postmortem for histopathologic detection of oxalate crystals; kidney and urine may be saved for possible analysis of EG or acidic metabolites.

Analysis • EG in serum or blood can be detected using a commercial test kit.* Results are best if analysis is performed less than 24 to 36 hours post-ingestion. Urine, if available, contains EG early (i.e., < 6 hours post-ingestion), followed by oxalates and calcium oxalate crystals, which can be viewed by light microscopy using polarized light. Calcium oxalate crystalluria (Color Plate 5D) appears 3 to 6 hours post-ingestion and, although not infallible, is useful for diagnosis. Serum calcium may be depressed after 4 hours because of complexing with oxalate metabolites of EG. Osmolality, osmolar gap, and anion gap are usually increased; severe metabolic acidosis is expected (see Chapter 6).

Normal Values • Trace amounts of EG or oxalate crystals.

Danger Values • EG values greater than 20 to 50 mg/dl indicate exposure; toxic concentrations vary widely, depending on initial dose and time since exposure. High anion gap and

*Ethylene Glycol Test Kit, PRN Pharmacal Inc., Pensacola, FL.

a severe metabolic acidosis suggest serious EG toxicosis.

Artifacts • Ethanol or methanol may also abnormally increase serum osmolality but should not cause oxalate crystals or high EG concentrations. EG kits cross-react with propylene glycol, formalin, glycerol, excessive serum LDH, and some drugs (e.g., pentobarbital, diazepam).

Causes • EG toxicity needs to be differentiated from toxic (see Tables 17-1 and 17-6) and other causes of acute CNS depression, metabolic acidosis, and oliguric renal failure. Rapid progression (i.e., 1 to 3 days) from CNS signs to oliguric renal failure with calcium oxalate crystals and metabolic acidosis with increased anion gap (see Chapter 6) allow a presumptive diagnosis of EG toxicity. CNS signs are transient and may be missed. Unless EG toxicity is treated within hours of ingestion, prognosis is poor.

Lead

Indications • Lead toxicity should be considered in dogs with periodic episodes of vomiting, with or without mild anemia and basophilic stippling, and cats with anorexia and occasional vomiting. Most lead-poisoned animals also experience seizures and behavioral changes (e.g., hysteria, depression) and occasionally ataxia and facial tremors.

Sample Collection • Whole blood collected in EDTA or heparin is the preferred antemortem sample and can be used to make blood smears looking for basophilic stippling and nucleated RBCs, to test for porphyrins (plasma), or for analysis for lead (lead is primarily in erythrocytes). Lead is stable in blood for long periods, but the sample should be refrigerated to preserve RBC integrity. Urine may be helpful (porphyrins are elevated). Liver and kidney are recommended for postmortem analysis.

Analysis • Lead is usually detected by atomic absorption spectroscopy analysis.

Normal Values • Less than 0.05 ppm in whole blood or less than 3 ppm in liver or kidney is considered nontoxic exposure. Normal range of urinary or plasma porphyrins is not well established in small animals. Normal human protoporphyrin values are less than 50 mg/dl. If submitted for analysis, the clinician should include specimens from a normal, unexposed animal for comparison.

Danger Values • Blood lead values greater than 0.3 ppm suggest excessive exposure, and greater than 0.4 ppm are usually considered confirmatory of toxicosis. Liver or kidney lead values greater than 10 ppm support a diagnosis of lead toxicosis.

Artifacts • Some laboratories that use organic chelate extraction of lead rather than acid digestion prefer heparinized whole blood. Above a minimum value, lead concentrations are not well correlated with severity of clinical signs but are a reflection of body burden. Values as little as 50% of the danger values (i.e., 0.15 ppm) could indicate toxicosis if other parameters (i.e., clinical signs, basophilic stippling, nucleated RBCs, elevated urinary or plasma porphyrins) are consistent with toxicosis.

Causes • Basophilic stippling is neither sensitive nor specific for lead poisoning. When basophilic stippling is found in conjunction with nucleated RBCs and absence of reticulocytosis, a lead determination is indicated. Lead poisoning must be differentiated from toxic (see Table 17-1) and other causes of CNS stimulation. Poisoning is most common in young dogs (i.e., < 1 year) with access to old housing, peeling paint, and lead objects. Blood lead concentrations establish exposure and usually correlate with toxicosis but not degree of severity. To detect prolonged low-level exposure with a relatively low blood lead concentration, the clinician should collect a urine sample, inject calcium disodium EDTA (75 mg/kg intramuscularly [IM]), and obtain a second urine sample 24 hours later. The clinician should measure urine lead concentrations both before and after EDTA administration; lead-poisoned animals should demonstrate greater than tenfold increase in urine lead. Ideally, 24-hour urine samples should be obtained.

Metaldehyde

Common Indications • Intoxication with metaldehyde should be considered in patients with acute onset of salivation, tremors, ataxia, seizures, nystagmus, and vomiting in conjunction with exposure to snail baits or solid fuels (i.e., solid blocks used for camp stoves, also known as “canned heat”).

Sample Collection • Samples can be collected in whole blood, serum, and urine and should be kept frozen until analysis. Postmortem samples (i.e., gastric contents, urine) establish exposure; toxic concentrations are not established.

Analysis • A quick test for metaldehyde is to place the bait in a test tube and warm it slowly. Metaldehyde present sublimes, then forms a substantial "snowfall" of crystals. Metaldehyde is metabolized to acetaldehyde, which can be detected by gas-liquid chromatography in serum or urine during acute poisoning. Acetaldehyde produces metabolic acidosis (see Chapter 6).

Normal Values • Metaldehyde is not expected in blood of normal animals.

Danger Values • Not documented.

Artifacts • Acetaldehyde is also a metabolite of ethanol. Acetaldehyde may form during analytic procedures unless blood proteins are precipitated before analysis.

Causes • Metaldehyde toxicosis is somewhat seasonal and geographically influenced. Mild, moist climates (e.g., West Coast, southeastern United States) are expected regions of metaldehyde exposure. Careful history correlated with clinical signs and detectable metaldehyde may allow a presumptive diagnosis. Metaldehyde must be differentiated from toxic (see Table 17-1) and other causes of CNS excitation.

Organophosphate and Carbamate Insecticides

Indications • Intoxications with organophosphates (OP) or carbamate should be considered in patients with muscarinic signs (i.e., salivation, gastrointestinal hypermotility, miosis), nicotinic signs (i.e., muscle tremors, weakness), and CNS excitation or depression, especially if previously treated with OP or carbamate compounds.

Sample Collection • For acethylcholinesterase (AChE) determination, the clinician should collect whole blood in EDTA or heparin. Samples should be refrigerated, not frozen. AChE is stable for at least 3 days when refrigerated; freezing may destroy or reduce the activity. Early sampling is important for carbamates because AChE may spontaneously reverse as soon as 1 to 3 hours after exposure. Sampling time is less critical with OP compounds, because they bind strongly after a period of time (known as *aging*). Because blood may not contain adequate OP or carbamate residues, the clinician should collect urine, vomitus, and potential baits or sprays for analysis as well. Fur or hair may be submitted if exposure is by dips or spraying. For dead animals, the clinician should submit the whole brain or at least the caudate nucleus (below the anterior ventral wall of lateral ventricles). *Caution:* Some OP and carbamate insecticides are highly toxic; clinicians should avoid self-exposure by wearing gloves or other protective clothing.

Analysis • Direct chemical analysis of OP or carbamate compounds is performed on baits, pesticides, stomach contents, and brain via gas-liquid chromatography. Serum pseudocholinesterase determination is inaccurate. Although residues of OPs and carbamates may be in blood or tissues, very low values and rapid metabolism often hinder detection. Most OP and carbamates are rapidly metabolized and may be undetectable in blood or tissues within minutes to hours after exposure. Determination of AChE activity is commonly used to detect activity of OP compounds. Several laboratory methods are used.

Normal Values • Different methods of analysis for AChE are used. The clinician should ask the laboratory for a range of normal values and to determine the percentage of inhibition. Treatment for OP toxicosis may reduce AChE by up to 50% within the first 1 to 7 days after treatment.

Danger Values • If whole blood AChE is greater than 50% depressed, excessive OP exposure is indicated. Significant OP or carbamate concentrations vary with the agent suspected. Usually, greater than 50% inhibition of AChE signifies overexposure, and greater than 75% inhibition supports a tentative diagnosis of toxicosis.

Artifacts • AChE values are highly variable. Blood values may be elevated by hepatic disease. In OPs, cholinesterase depression is persistent; in carbamates, cholinesterase values quickly return to normal.

Causes • Typical clinical signs, depression of cholinesterase, and detection of pesticide is

usually adequate for diagnosis. Other toxins (see Tables 17-1 and 17-2) and other causes of CNS excitation or depression, vomiting, and diarrhea (see Chapter 9) need to be considered. The triad of muscarinic, nicotinic, and CNS signs is similar to pyrethrin intoxication (discussed next).

Pyrethrin and Pyrethroid Insecticides

Indications • Intoxication with pyrethrin and pyrethroid insecticide should be considered in animals with tremors, salivation, vomiting, or CNS excitation or depression after application of dips or sprays. Toxicosis is rare unless oral or respiratory exposure has taken place.

Sample Collection • Antemortem animal samples are not useful because of analytic difficulties and interpretation. Baits or sprays can be analyzed for comparison with the stated label concentration. Hair or fur may be used to establish exposure. Stomach contents and brain have been used occasionally. Refrigeration or freezing is adequate preservation.

Analysis • Analysis is accomplished through gas-liquid chromatography of concentrated sources such as sprays, dips, hair or fur, or stomach contents. Tissues from exposed animals usually contain very low concentrations. Analysis of brain has been used to confirm exposure, but quantitation correlating with toxicosis is rare. AChE is not inhibited by pyrethrins, so AChE determination can help eliminate OP toxicosis.

Normal Values • Pyrethrins are not expected in tissues of normal animals.

Danger Values • Not well documented. Presence of pyrethrins indicates exposure, which must be correlated with historical and clinical evidence. Concentrations of several hundred ppm could be present in the stomach or on hair and only indicate exposure, whereas more than 100 ppb in brain could suggest toxicosis.

Causes • Because of the safety of these products with normal use, pyrethrin toxicosis is more often suspected than proven. Most reported poisonings are in cats. Analyses for other toxicants (e.g., OPs, carbamates) are used to help rule them out and establish exposure to pyrethrins.

Vitamin D

Indications • Intoxication with vitamin D should be considered in animals exposed to compounds containing vitamin D_3 (i.e., cholecalciferol). Common trade names include Quintox, Rampage, and Ortho-Rat-B-Gone. Baits usually contain 0.075% cholecalciferol. Clinical signs occur after a 1- to 2-day latent period and include anorexia, lethargy, weakness, and vomiting.

Sample Collection • Because of the lag period between consumption and onset of clinical signs, time of collection of stomach contents is critical to interpretation (see Analysis). Serum or plasma (EDTA or heparin anticoagulant) can be analyzed. The sample should be refrigerated before analysis and frozen if analysis will be delayed more than 24 hours. Marked hypercalcemia (i.e., >14 mg/dl; see Chapter 8) is highly suggestive of toxicosis, and testing for hypercalcemia is easier than analysis for cholecalciferol or its metabolites. At postmortem, liver and kidney should be collected for potential analysis of vitamin D metabolites.

Analysis • Baits can be tested for vitamin D_3. Stomach contents or vomitus may contain small amounts (i.e., < 0.01%) of cholecalciferol. Because of the latent period, the stomach has often emptied the toxic material. Although liver and kidney contain vitamin D metabolites (e.g., 1,25 dihydroxy cholecalciferol), testing is specialized (gas-liquid chromatography, high-performance liquid chromatography, or both) and not routinely offered by clinical laboratories. Histopathologic examination with special stains for calcium may also aid in detection of renal or vascular calcification. Postmortem analysis reveals elevated renal calcium values.

Normal Values • Serum 1,25 dihydroxycholecalciferol values less than 30 pg/ml are considered normal. Normal canine renal calcium values are 50 to 200 ppm on a wet weight basis.

Danger Values • Serum 1,25 dihydroxycholecalciferol values greater than 30 pg/ml indicate potential toxicosis, and values greater than 50 pg/ml are toxic. Postmortem renal calcium concentrations greater than 2000 ppm and less than 8000 ppm support a diagnosis of cholecalciferol toxicosis. Values above 8000 ppm indicate potential EG toxicosis.

Artifacts • Hyperactivity of parathyroid function or tumors associated with hypercalcemia (e.g., lymphosarcoma) can produce serum calcium values similar to those of cholecalciferol toxicosis (see Table 8-1).

Causes • A tentative diagnosis can be established from history, clinical signs, hypercalcemia, and azotemia. Other common causes of hypercalcemia are discussed in Chapter 8.

Zinc

Indications • Intoxication with zinc should be considered in patients with acute vomiting, hematuria, hemoglobinuria, icterus, renal failure, and hepatic failure. Radiography showing radiopaque objects (e.g., pennies) in the gastrointestinal tract is an indication for further testing.

Sample Collection • Either whole blood (i.e., EDTA, heparin anticoagulant) or serum may be used for analysis. The clinician should consult a laboratory to determine preferred specimens. Trace element-free tubes are preferred (i.e., Vacutainer, royal-blue stopper). Refrigeration is adequate preservation. Postmortem samples include liver and kidney.

Analysis • Zinc analysis is performed by atomic absorption spectroscopy.

Normal Values • Less than 2.0 mg/ml in serum. Normal liver zinc is 30 to 70 ppm (wet weight basis).

Danger Values • During toxicosis, serum zinc may range from 3 to 10 µg/ml. Liver and kidney zinc concentrations greater than 200 ppm (sixfold to eightfold increase over normal) are likely during toxicosis.

Artifacts • Plastic syringes with rubber grommets may contain zinc and contaminate a sample.

Causes • Most reported cases are from ingestion of zinc-containing objects (e.g., pennies). Other causes of hemolytic anemia (see Chapter 3), renal failure (see Chapter 7), and vomiting (see Table 17-2 and Chapter 9) need to be eliminated. Radiography is indicated in dogs with vague signs of vomiting, hepatic or renal disease, and hemolytic anemia. Vomiting, laboratory indicators of hepatic damage (e.g., increased ALP and ALT), azotemia, and intravascular hemolysis (especially if a gastric metal density is observed on abdominal radiographs) are highly suggestive of zinc toxicosis.

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Dawn Merton Boothe

Therapeutic Drug Monitoring

Fixed dosing regimens provided on drug labels are designed to generate plasma drug concentrations (PDCs) within a therapeutic range (Figure 18-1). Plasma drug concentrations are intended to remain above a minimum effective concentration (C_{min}) to avoid therapeutic failure while remaining below the maximum concentration (C_{max}) and minimizing side effects. Fixed dosing regimens are based on pharmacokinetic studies conducted in a small sample population of normal adults. These regimens, however, are generally administered to unhealthy animals for which drug absorption, distribution, metabolism, or excretion (or a combination of these) have been altered by physiologic factors (e.g., age, gender), pathologic factors (leading to renal or hepatic impairment), or pharmacologic factors (e.g., drug interactions). As a result, PDC may be higher or lower than expected. In these instances, individual monitoring and adjustment of doses using therapeutic drug monitoring (TDM) can optimize drug efficacy and safety. Drugs for which TDM has proven useful in veterinary medicine include selected anticonvulsants (e.g., phenobarbital, primidone, potassium bromide, selected benzodiazepines); antimicrobials (e.g., aminoglycosides: gentamicin, amikacin); cardioactive drugs (e.g., digoxin, procainamide, lidocaine); theophylline, thyroid hormones (for thyroid supplementation); and, more recently, cyclosporine (Table 18-1).

Indications • TDM may be indicated whenever patients fail to respond appropriately to a drug or when the risk of drug toxicity is great. Specifically, TDM is most useful in six situations when (1) the clinical end point of drug therapy is poorly defined or difficult to detect (e.g., anticonvulsant therapy); (2) the therapeutic index is narrow, indicating little difference exists between effective and toxic PDC (e.g., digoxin, theophylline); (3) marked interindividual pharmacokinetic variability exists, making it difficult to predict PDCs (e.g., phenobarbital); (4) pharmacokinetics are nonlinear, leading to rapid accumulation of toxic concentrations (e.g., phenytoin, phenobarbital [in cats]); (5) drug interactions potentiate toxicity (e.g., enrofloxacin-induced theophylline toxicity, chloramphenicol- or clorazepate-induced phenobarbital toxicity); or (6) when the disease

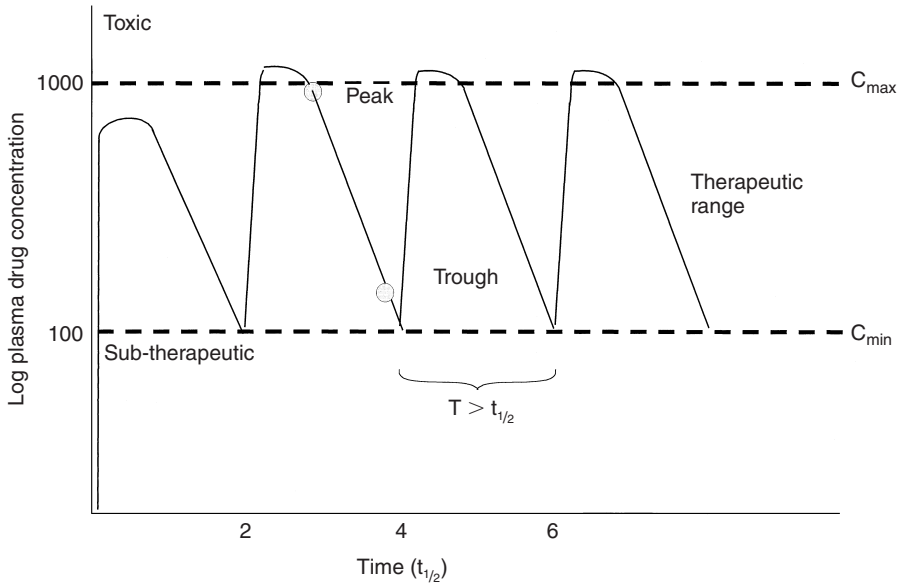

FIGURE 18-1. Plasma drug concentrations (PDCs) after multiple administration of a drug with a half-life that is shorter than the dosing interval. In this example, the dose is administered every four half-lives. Because most (94%) of each dose is eliminated before the next dose, PDCs fluctuate markedly. Both toxic and subtherapeutic drug concentrations can occur during a single dosing interval; both peak and trough samples should be collected for such drugs.

is life-threatening, and administration of large doses of drugs is necessary to achieve a prompt response (e.g., epilepsy, bacterial sepsis). Occasionally, TDM is used to identify owner noncompliance as a cause of therapeutic failure or adverse drug reaction, or when overdose may have occured.

ANALYSIS AND LABORATORY AVAILABILITY

Generally, PDCs are measured with assays involving specific binding of antibodies to the drug of interest. These antibody-based methods are easily automated and are usually more rapid and cost-effective than chromatographic and spectrophotometric assays, the latter being necessary for a limited number of drugs. With the exception of assay of bromide, which should always be analyzed using the gold chloride method (until the ion-sensitive electrode method is validated), a variety of assay methods can be used to accurately measure PDCs of most drugs.

TDM is offered at most veterinary colleges and many diagnostic laboratories in the United States. However, the range of services offered and methods of analysis used vary widely. Before one submits samples to a particular laboratory, information should be sought regarding procedures for sample handling, delivery, and quality assurance practices. It is imperative that each assay is validated for use in the species of interest and that results generated by the laboratory are accurate and reproducible. When submitting samples to facilities usually serving human patients, one must be cautious not to confuse recommended therapeutic ranges in humans with those in animals (e.g., clorazepate, bromide, procainamide). Among the more critical considerations is the availability of recommendations by a clinical pharmacologist.

ARTIFACTS

Assuming proper quality assurance practices are used, false assay results arising from errors related to drug analysis are rare. A notable exception may be for drugs that are metabolized. If the metabolites are active (e.g., clorazepate), the assay should measure all active compounds. If the metabolites are inactive or only weakly active (e.g., cyclosporine), the assay should be specific for the parent compound only (e.g., phenobarbital)

TABLE 18-1. Therapeutic Drug Monitoring Data for Drugs Monitored in Normal, Healthy, Small Animals

DRUG	USUAL DOSAGE	INTERVAL (HR)	THERAPEUTIC RANGE*	ELIMINATION HALF-LIFE	TIME TO STEADY STATE	SAMPLE COLLECTION PEAK	SAMPLE COLLECTION TROUGH
Amikacin	15-20 mg/kg	24	2-25 µg/ml[1]	1-2 hrs	<1 day	1 hr (plastic only)	Two half-lives (3 to 6 hrs)[2]
Aspirin							
(dog)	10 mg/kg	8-12	50-100 µg/ml	8 hrs	40 hr	2-4 hrs	BND
(cat)	10 mg/kg	72	50-100 µg/ml	38 hrs	8 days		
Benzodiazepines	1-2 mg/kg		100-200 ng/ml[3]	<8 hrs	1 day	2-5 hrs	BND[2]
Bromide	15-45 mg/kg	12-24	1.0-3.5 mg/ml	24 days	2-3 months		BND
Cyclosporine	High: 6.0-8.5 mg/kg	12	Trough above	5.6 hrs	<1 day[4]	2-4 hrs	BND
	Moderate: 3.5-5.5 mg/kg	12	400 to 600 ng/ml				
	Low: 0.75-3.00 mg/kg	12					
Digoxin							
(dog)	0.011 mg/kg	12	0.9-3.0 ng/ml	31.3 hrs	7 days	Toxicity: 2-5[5] hrs	Efficacy: BND[5]
(cat)	0.008 mg/kg	12-24	0.9-2.0 ng/ml	33.5 hrs	7 days	(glass only)	
Gentamicin							
(dog)	2-8 mg/kg	12-24	0.5-1.5 µg/ml[1]	0.9-1.3 hrs	<1 day	1 hr (plastic only)	Two half-lives (3-6 hrs)[2]
(cat)	2-8 mg/kg	12-24	5.0-8.0 µg/ml				
Phenobarbital							
(dog)	2 mg/kg	12	20-45 µg/ml	32-75 hrs	14-16 days	4-5 hrs[6]	BND
Primidone							
(dog)	11-25 mg/kg	12-24	Based on phenobarbital	6.1 hrs (D)	14-16 days	4-5 hrs[6]	BND
(cat)	11-20 mg/kg	12-24					
Procainamide							
(dog)	15 mg/kg	12	25-50 µg/ml[7]	2.9 hrs	<1day (15 hrs)	2-4 hrs	BND
Theophylline							
(dog)	7-11 mg/kg or 20 mg/kg slow release	8-12; slow release: 12	10-20 µg/ml	5.7 hrs	29 hrs	1-2 hrs[8]	BND
(cat)	4 mg/kg or 20 mg/kg slow release	12-24; slow release: 24	10-20 µg/ml	7.9 hrs	40 hrs		

Thyroid hormones	T3: 4-6 µg/kg (D)	12-24	0.8-1.5 ng/ml (D)[9]	5-6 hrs (D)	<24 hrs[11]	4-5 hrs	BND
	4.4 µg/kg (C)	12-24	0.8-1.5 ng/ml (C)[9]				
	T4: 20 µg/kg (D)	12-24	1.5-3.5 µg/dl (D)	12-15 (D)	48-72 hrs[11]		
	50-100 µg/kg(C)	12-24	1.5-5.0 µg/dl (C)[10]				

BND, Before next dose; *C*, cat; *D*, dog.

*Therapeutic ranges are extrapolated from human patients unless noted otherwise. Ranges may also vary with the laboratory findings and specifically with the instrumentation used to assay the drug of interest. Values in this table may be superseded if the values for the instrument have been appropriately validated. Because samples sizes and assay methodologies vary, the specific laboratory that performs the assay should be contacted regarding sample volume, proper collection tubes, need of refrigeration, and other sample handling specifics, as well as "normal" ranges.

[1]"Target" peak concentration for aminoglycosides depend on the infecting organism and specifically on the minimum inhibitory concentration (MIC) of the infecting organism. The target peak concentration should be 4 to 10 times the MIC. Trough concentration should be equal or below that recommended to minimize toxicity.

[2]For drugs with a very short half-life and at low concentrations, trough sample may no longer have detectable drug. Wait one or two predicted elimination drug half-lives between peak and trough sample collections.

[3]600 ng/ml listed in humans. Assay should measure all benzodiazepines (parent and active metabolites) relative to dosing interval. If loading, single sample immediately post-load and 3 weeks later. If not loading, single sample at 3 to 4 weeks. For either, collect single sample at 3 months for new baseline.

[4]In people; data limited in dogs. Trough of 100 ng/ml may be acceptable for some indications. Concentrations also may vary with assay. Laboratory should be contacted for specific range for their assay.

[5]Both peak and trough recommended because of short half-life; single peak acceptable if toxicity is a concern.

[6]Peak and trough recommended if seizures are difficult to control.

[7]As suggested in Papich MG, Davis LE, Davis CA: Procainamide in the dog: antiarrhythmic plasma concentrations after intravenous administration, *J Vet Pharmacol Ther* 9:359, 1986.

[8]For slow release preparations, one sample may be sufficient.

[9]Values for ranges of thyroid hormones reflect an RIA assay. Values are likely to be different for each laboratory. Contact the laboratory, or if doing in house, establish your own normal ranges. Overlap between normal and abnormal is great, regardless of the laboratory and interpretation should be based on clinical signs.

[10]Monitoring can occur at any time for cats in whom hyperthyroidism is being managed.

[11]Monitoring should not take place until the body has had a chance to physiologically adapt to drug therapy (i.e., 4 to 6 weeks after therapy is implemented).

or the therapeutic range of the assay should reflect the metabolites. Thus the therapeutic range of a drug may vary depending on the assay performed. Erroneous PDC data are more likely to be caused by inappropriate sample collection. Serum separator tubes should be avoided because silicon gel in the tubes can bind to and remove drug from the sample, leading to falsely decreased values. Other examples of artifacts caused by collection tubes include binding of aminoglycosides to glass tubes and binding of digoxin to red stoppers. Hemolysis of blood samples and lipemia should be avoided, but their impact is negligible.

IMPLEMENTING THERAPEUTIC DRUG MONITORING

Handling Procedures and Decisions

In general, serum is monitored; however, most methods can measure drug concentrations in plasma. Cyclosporine should be measured in whole blood. If samples are to be assayed immediately, special handling procedures (e.g., refrigeration, freezing) often are not necessary. Before TDM is conducted, several decisions should be addressed. Three of these are (1) when TDM should be initiated, (2) the number of samples to be collected, and (3) the timing of sample collection. Because of variability among laboratories, the laboratory of submission should be contacted regarding sample submission.

Steady State

When therapy is initiated, repeated and regular administration of successive doses may result in accumulation of drug. The greater the half-life compared with the dosing interval, the greater the amount of drug accumulation. Eventually the amount of drug eliminated during each dosing interval equals the amount administered with each dose. At this time, steady state has been reached; peak and trough blood concentrations remain constant as long as dose and dosing interval are not changed. Ideally, TDM and clinical response to therapy should be implemented at steady state to assess maximum response. The time taken for steady state to be reached depends only on the rate of drug elimination (i.e., drug elimination half-life), irrespective of dose or interval. With multiple dosing of drugs that accumulate, PDC reaches 50% of steady-state concentrations at one half-life, 75% by two half-lives, 87.5% at three half-lives, and so forth (Figure 18-2). Generally, TDM should not be implemented until three to five half-lives have elapsed since initiation of drug therapy. For example, for phenobarbital with a $t_{1/2}$ of 72 hours, TDM should not be implemented until approximately 15 days after initiation of therapy. If any aspect of the dosing regimen is changed, the same time period (i.e., five half-lives) must elapse for steady-state plasma concentrations to be reestablished. An exception might be made for bromide, for which a sample might be taken at one half-life (i.e., 3 weeks) after a dose is begun to proactively assess a dosing regimen. At one half-life, drug concentrations should approximate the steady-state concentration. Times taken to achieve steady state (as well as other pharmacokinetic and dosing information) are listed in Table 18-1. For drugs with very short $t_{1/2}$ values compared with the dosing interval (e.g., gentamicin $t_{1/2}$ = 0.9 to 1.3 hours; dosing interval 12 to 24 hours), steady state is irrelevant, because the drug does not accumulate with repeated dosing. As such, TDM can be initiated immediately (i.e., after the first dose).

Loading Dose

When the clinical situation necessitates immediate attainment of therapeutic drug concentrations (e.g., a seizuring patient being treated with bromide), PDC predicted at steady state can be achieved more rapidly by administration of a single loading dose followed by recommended maintenance doses (see Figure 18-2). Loading doses are based on the volume of tissue that dilutes the drug and, for drugs not administered intravenously (IV), the bioavailability of the drug. Despite the fact that steady-state PDCs have been achieved by loading, PDCs are not yet at steady state. PDC achieved after loading may increase or decrease if the maintenance dose is more or less, respectively, than that eliminated during each dosing interval. For bromide in particular, TDM should occur within 1 to 3 days after loading to confirm that targeted concentrations (i.e., predicted steady-state concentrations) have been achieved and again one drug half-life later (i.e., 3 to 4 weeks for bromide) to ensure that the maintenance dose is maintaining the PDC achieved with loading. Although use of a loading dose decreases the time taken for maximum

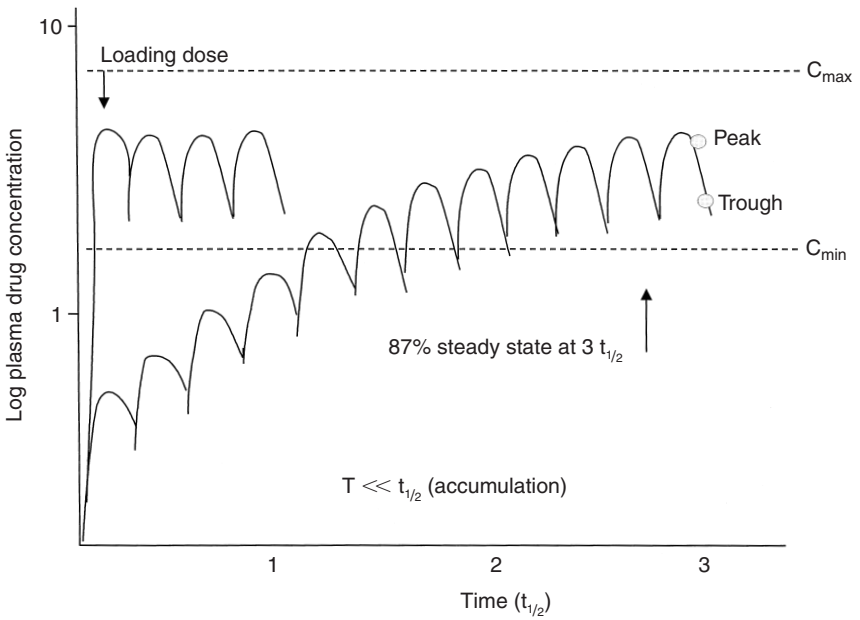

FIGURE 18-2. Plasma drug concentrations (PDCs) after multiple administration of a drug with a half-life that is longer than the dosing interval. In this example, the dose is given approximately twice every drug half-life. Because little of each dose is eliminated during the dosing interval, little fluctuation occurs and a single sample can be collected for monitoring. To rapidly achieve a pharmacologic effect, a loading dose might be given. A steady-state equilibrium will still occur, however, and PDCs may increase or decrease if the maintenance dose does not maintain what the loading dose achieved.

response to occur (by avoiding slow accumulation to steady state), hazards of adverse reactions are much greater. Thus loading doses are not advised for drugs characterized by a narrow therapeutic index and that tend to cause undesirable adverse reactions (e.g., digoxin).

Number of Samples

The relationship between drug half-life and dosing interval (as well as the intent of monitoring) determine the number of samples to be collected. If the dosing interval is longer than drug half-life (e.g., diazepam, most antibiotics), the PDC fluctuates widely during each dosing interval (see Figure 18-1). Assuming that both efficacy and safety are of interest, one should collect two samples to coincide with peak and trough concentrations. For drugs with a long half-life compared with dosing interval, drug concentrations do not change much during each dosing interval, and a single sample generally reflects PDC throughout the dosing interval. For digoxin, collection of both a peak and trough sample is encouraged because of the narrow therapeutic index of this drug and the marked variability in half-life that can occur in the patient receiving cardiovascular drugs. A single trough sample is often sufficient for phenobarbital. However because elimination half-lives of less than 24 hours have been measured, clinicians should collect both a peak and trough sample in patients in which seizure control is difficult.

Timing of Sample Collection

Timing for single sample collection depends on the intent of TDM. If efficacy is of concern and only one sampling time is indicated, assessment of trough concentrations is recommended (because these can consistently be compared with subsequent TDM). One can easily determine the trough concentration by collecting the sample immediately before administration of the next dose. Ideally, trough PDC will not drop below recommended C_{min}. For drugs with a short half-life compared with the dosing interval (e.g., the aminoglycosides) and for which both

a peak and trough sample will be submitted, collection of a trough sample may result in nondetectable concentrations. Collection of a trough sample at 2 to 3 half-lives after dosing would be more prudent for such drugs. For toxicity, a single peak sample should be determined.

Timing of samples used to determine peak concentrations is more difficult to estimate because these samples should only be collected when drug absorption and distribution are complete. In particular, oral absorption of drugs is variable and is influenced by feeding (fasting is generally indicated). Generally, peak PDCs occur 2 to 4 hours after oral administration, although drugs that are absorbed slowly take longer to achieve peak PDC (e.g., 5 hours for phenobarbital). For intramuscular (IM) and subcutaneous (SC) administrations, absorption occurs more rapidly (i.e., 30 to 60 minutes). Absorption times are not relevant to IV-administered drugs, but distribution may take 1 to 2 hours. Thus peak PDCs are generally measured 1 to 2 hours after parenteral drug administration. Peak and trough samples are generally collected during a single dosing interval.

MODIFYING DOSE REGIMENS

Precise adjustment of dose regimens requires generation of a pharmacokinetic profile and calculation of pharmacokinetic parameters, such as clearance values and volumes of distribution. If this degree of sophistication is necessary, a specialist in clinical pharmacology should be consulted. A modified profile can be generated from data collected with two (peak and trough) samples. For situations in which precise dose adjustment is not essential and the patient is at steady state, a relatively simple approach is to use the direct proportionality between dose and PDC. For example, if the dose is doubled, the resulting PDC will also be doubled. Conversely, if the dose is halved, the resulting PDC will be halved. The following dose adjustment equation describes this relationship:

$$\text{New dose} = \frac{\text{Old dose} \times \text{Target PDC}}{\text{Measured PDC}}$$

With the therapeutic ranges listed in Table 18-1 and PDC results derived from TDM, a new adjusted dose can be calculated that will achieve a desired target PDC. For example, if the measured peak PDC after administration of 0.011 mg/kg of digoxin to a dog is 0.5 ng/ml and a decision is made to increase the dose to achieve a target peak PDC of 1.5 ng/ml, the new dose can be estimated as follows:

$$\text{New dose} = \frac{0.011 \text{ mg/kg} \times 1.5 \text{ ng/ml}}{0.5 \text{ ng/ml}} = 0.033 \text{ mg/kg}$$

Increasing the dose for drugs with a short half-life may result in PDCs that are both too high and too low during a dosing interval. The decision to modify the dose versus interval should take into account client convenience and desired fluctuation in PDC during the dosing interval. The dosing interval also can be changed proportionately, although calculation of elimination half-life provides a more accurate method on which changes in dosing interval can be based. The elimination half-life of a drug can be calculated as $t_{1/2} = 0.693/\text{kel}$, where kel is the slope of the line drawn between the two TDM points (peak or C_1, t_2; and trough, C_2, t_2): kel = ln $[C_1/C_2]$ divided by $t_2 - t_1$. The natural log (ln) must be used because drug elimination is first-order. A clinical pharmacologist can be consulted for assistance in the design of a dosing regimen.

The clinician should never base decisions to modify a dosing regimen solely on PDCs and their relationship to the therapeutic range. The therapeutic ranges that accompany TDM results are not the same as "normals" that accompany clinical laboratory tests. A therapeutic range reflects the C_{min} and C_{max} between which a large percentage (e.g., 95%) of the target population responds to drug therapy. Some animals, however, respond outside the therapeutic range (below C_{min} or above C_{max}), whereas other animals may become "toxic" within the therapeutic range. Clinical response always must be considered when doses are adjusted. This is particularly important for drugs with effective therapeutic and toxic PDC ranges that overlap (e.g., digoxin, thyroid hormones). Whenever doses are changed, TDM should be continued to confirm that target PDCs have been achieved.

When PDCs are within the recommended therapeutic range but the animal fails to respond satisfactorily, minor, "stair step" adjustments in dose are recommended. The size of each increment depends on the

therapeutic range and safety of the drug. If a patient's response to the drug is insufficient, doses are increased proportionately to the desired increase in PDC until either desired response is achieved or maximum limit of the range is reached and risk of adverse effects precludes further increase in dose. For example, phenobarbital (therapeutic range: 15 to 45 μg/ml) should be increased by 5 μg/ml and bromide (therapeutic range: 1.0 to 3.5 mg/ml) by 0.5 mg/ml increments. Doses for each drug are increased by about 25% to achieve the incremental increase. Likewise, if PDCs are close to the maximum of the range (raising concerns that toxicity could result), stepwise decreases can be used to establish the minimum effective concentration necessary to control clinical signs while avoiding toxicity (e.g., anticonvulsant therapy). For each dose change, response should not be evaluated until a new steady state has been reached.

THERAPEUTIC MONITORING OF SELECTED DRUGS

Aminoglycosides (Amikacin, Gentamicin)

Indications • To monitor aminoglycoside PDCs in life-threatening, serious, or chronic infections caused by susceptible bacteria.

Sample Collection • Generally, both C_{max} (to verify efficacy) and C_{min} (to verify safety) are indicated. Current dosing recommendations are for 24-hour dosing intervals. Ideally, C_{max} should be 8 to 10 times the minimum inhibitory concentration of the drug (based on culture and susceptibility data). C_{min} concentrations should be less than 2 μg/ml. Because the elimination half-life of the aminoglycosides is short (i.e., 1 to 3 hours), trough concentrations should not be collected just before the next dose because it is unlikely that concentrations will still be detectable. Rather, trough aminoglycoside concentrations should be collected at 4 to 6 hours after peak concentration.

Artifacts • Aminoglycosides are bound to glass. Samples intended for TDM should not be collected in glass tubes or should be immediately transferred upon collection to plastic tubes.

Dosing Modification • Doses can be proportionately increased or decreased based on C_{max}. Intervals should be prolonged in increments of one drug half-life if C_{min} is above recommended trough concentration (see Table 18-1).

Benzodiazepines (Diazepam, Clorazepate)

Indications • To make sure PDCs do not drop below C_{min} in patients receiving the drug for long-term seizure control. Diazepam is generally measured in cats and clorazepate in dogs. Because each is metabolized to active metabolites, both parent drug and metabolites are monitored.

Sample Collection • The elimination half-life of the benzodiazepines is short, and PDCs fluctuate dramatically during an 8-hour or 12-hour dosing interval. Two samples are recommended (see Table 18-1).

Dosing Modification • The interval should be decreased if PDCs markedly fluctuate during the dosing interval. The dose should be changed proportionately as indicated by PDCs. Both the dose and interval may be modified simultaneously.

Bromide

Indications • Any epileptic patient receiving the drug. Regardless of the salt used, bromide is the active ingredient measured.

Sample Collection • The half-life of bromide is very long compared with the dosing interval; hence, single samples are indicated for TDM. Trough concentrations are recommended, although any time during the dosing interval is acceptable. Concentrations should be measured at baseline steady state, at 3- to 6-month intervals, and any time the animal has a seizure. Proactive monitoring should occur after a loading dose and at 1 month post-load. If a loading dose is not administered, monitoring should occur 1 month into therapy. A clinical pharmacologist is strongly recommended for consultation when monitoring bromide.

Artifacts • Only one method using gold chloride has been validated for monitoring bromide in serum. As mentioned previously, until the ion-sensitive electrode method is validated in serum, such methods should not be used for bromide analysis. Bromide can cause chloride concentrations to be artifactually increased.

Cyclosporine

Indications • In patients undergoing organ transplantation or in selected immune-mediated diseases. The drug has not been extensively used in dogs or cats and can cause toxicity. However, toxic concentrations have not been established in animals.

Sample Collection • Because the half-life is sufficiently short, both peak and trough concentrations should be collected (see Table 18-1). If a single sample is collected, a trough sample is indicated. Because the drug distributes to red blood cells (RBCs), whole blood generally should be collected.

Dosing Modification • Ideally, both dose and interval should be altered as necessary. If single samples are collected, the dose should be proportionally changed. The therapeutic range is generally based on trough samples and varies with the methodology and the disease being treated.

Digoxin

Indications • In patients suspected of digoxin toxicity or in patients with inadequate response.

Sample Collection • Both a peak and trough sample are necessary to design the safest and most effective dosing regimen, particularly in patients with an inadequate response or those in which disposition of digoxin is likely to be abnormal because of changes in renal and hepatic function induced by either disease or drug therapy. If toxicity is of concern, a single peak sample may be sufficient. Absorption and distribution of digoxin can vary with product used and the patient, and it can take up to 8 hours. Peak concentrations at 3 to 5 hours are recommended (see Table 18-1). The half-life of digoxin also varies, being as short as 12 hours in some animals despite a 36-hour half-life reported in normal animals. Thus, both a peak and trough sample are recommended. If a single sample is to be collected to evaluate efficacy, a trough sample should be collected. Because cardiac disease can cause marked disposition changes, TDM should be implemented before and after response to afterload therapy, diuretic therapy, or both because disposition is likely to change as the disease responds to therapy.

Artifacts • Red stoppers on collection tubes may bind drug, decreasing concentrations.

Dosing Modification • If a single sample has been collected, the dose of digoxin should be changed proportionately. If both peak and trough samples are collected, a pharmacokinetic profile (see earlier) should be generated for the patient and an interval appropriate and convenient should be established along with a new dose.

Phenobarbital and Primidone

Indications • Any animal receiving either of these drugs for seizure control. Primidone is converted to phenobarbital, which is measured rather than primidone. The disposition of both drugs varies markedly among animals. In addition, both drugs cause induction of drug-metabolizing enzymes, leading to greater variability. Finally, side effects (i.e., grogginess, hepatotoxicity) of the drug are of sufficient concern that samples should be monitored for safety.

Sample Collection • The half-life of phenobarbital can be short enough to necessitate both peak and trough samples, or it can be long enough to allow only a trough sample collection. Both peak and trough samples are recommended at baseline and, in anticipation of induction, 1 to 3 months later (see Table 18-1). If the patient remains seizure-free, a single trough sample is sufficient at 6-month intervals. However, if the patient has a "breakthrough" seizure, both peak and trough samples are recommended.

Procainamide

Indications • Although not routine, procainamide could be monitored in patients responding inadequately to long-term control of cardiac arrhythmias.

> NOTE: Efficacy of procainamide in people reflects an active metabolite that is minimally formed in dogs. Recommended concentrations reflect both procainamide and its acetylated metabolite.

Sample Collection • Because the half-life of procainamide is sufficiently short and the potential of adverse drug interactions sufficiently great, both peak and trough

samples should be collected (see Table 18-1). A single trough sample can also be used to verify efficacy.

Theophylline

Indications • In patients receiving the drug as a bronchodilator that have not experienced sufficient response and those suspected of reacting adversely to theophylline.

Sample Collection • The half-life of regular theophylline is short enough that it is best to collect both peak and trough samples (see Table 18-1). For efficacy, a single trough sample is acceptable. For safety (i.e., detection of adverse reactions), a single peak sample can be collected. For slow-release preparations, absorption is slow enough that a single trough sample is sufficient.

Dosing Modification • The dose of theophylline can be changed proportionately. Intervals can be modified if both peak and trough data are available.

Thyroid Hormones

Indications • In any animal receiving thyroid supplementation (usually dogs) or animals with hyperthyroidism that are being medically managed (usually cats). Because thyroxine (T_4) is the circulating hormone of interest (T_3 being located primarily intracellularly), it is generally the hormone measured. The majority of the hormone is bound to proteins and thus is pharmacologically inactive; therefore, free T_4 (fT_4) may be the preferred hormone to be tested. Equilibrium dialysis is the most accurate method to measure fT_4.

Sample Collection • Although concentrations of hormones may achieve steady state rapidly, the body may not equilibrate immediately. Physiologic equilibrium to the thyroid hormones may take 4 to 6 weeks, and monitoring should not take place until equilibrium is likely to have occurred (see Table 18-1). Likewise, response to drugs intended to control hyperthyroidism may take 3 to 4 weeks.

Analysis • Methods that have been validated in the species of interest are preferred.

Dosing Modification • Doses should be altered proportionately.

References

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Pippenger CF, Massoud N: Therapeutic drug monitoring. In Benet LZ et al, editors: *Pharmacokinetic basis for drug therapy*, New York, 1984, Raven Press.

Price CP: Analytical techniques for therapeutic drug monitoring, *Clin Biochem* 17:52, 1984.

Therapeutic drug monitoring, clinical guide, Abbott Laboratories, Diagnostic Division, Dallas, TX, 1984, Abbott Laboratories.

Wilson RC: Therapeutic drug monitoring, *Auburn Vet* 42(3):20, 1987.

Appendix I

Harold Tvedten and Michael D. Willard

Listing of Referral Laboratories

This appendix contains a noncomprehensive list of laboratories used by the clinical pathology laboratory of the Veterinary Teaching Hospital at Michigan State University and by some of the authors. Because the list includes referral laboratories that are mainly used by the veterinary college's laboratory, most of the entries are from the Animal Health Diagnostic Laboratory at Michigan State University. Readers are encouraged to identify a veterinary diagnostic laboratory nearest to them and obtain that laboratory's current price list and submission requirements (see disclaimer).

Readers should contact the laboratories they have selected for current, correct information concerning submission requirements, techniques, fees, and reference values before submitting samples. Note that these laboratories perform many more tests than are listed here. Under a specific test listing, only one or a few laboratories are arbitrarily included, although many laboratories may offer that test.

Most common clinical pathology tests are omitted, because most veterinary laboratories perform them. Specific clinical pathology tests are discussed in appropriate chapters. The information available from this listing often includes the name of the laboratory, service role, service range, address, phone number, and how certain types of samples should be submitted. Absent information was not readily available to the author.

DISCLAIMER

The list was included to provide some basic, practical information. There was no intent to advertise or certify these laboratories at the expense of unlisted laboratories, to create a comprehensive list of referral laboratories in North America, or to describe how to submit samples for all useful tests. This listing does not ensure that the laboratories will be able to process the readers' samples or that the information included is always current or appropriate. Fees are frequently changed. Submission procedures recommended by the particular laboratory for various tests may vary with those recommended in the rest of the book, and a laboratory may change its recommendations. This is especially true of endocrine tests.

ORGANIZATION OF THE LIST

The addresses and full description of laboratories are only listed once. Laboratories with multiple listings are described in the first section. Only a partial address of these laboratories is then given in the later alphabetic list of individual tests; therefore, readers must refer back to the alphabetic listing of these laboratories for addresses and phone numbers. The second section on individual tests is broken into subsections on endocrine, immunologic, serologic, toxicologic, and other tests. Laboratories listed with just one test have their address and other descriptions included with that test.

Arizona
Arizona Veterinary Diagnostic Laboratory
2831 North Freeway
Tucson, AZ 85705
Phone: 520-621-2356

California
California Veterinary Diagnostic Laboratory
PO Box 1770
Davis Branch Laboratory
University of California—Davis
Davis, CA 95617
Phone: 530-752-8700

Colorado
Colorado Veterinary Diagnostic Laboratory
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, CO 80523
Phone: 970-491-1281

Connecticut
Department of Pathobiology
University of Connecticut
Box U89
61 North Eagleville Road
Storrs, CT 06269-3089
Phone: 860-486-3736

Florida
Animal Disease Laboratory
Florida Department of Agriculture
PO Box 460
Kissimmee, FL 32742
Phone: 407-846-5200

Georgia
Diagnostic Laboratory
College of Veterinary Medicine
University of Georgia
Athens, GA 30602-7383
Phone: 706-542-5568

Veterinary Diagnostic Laboratory
University of Georgia
PO Box 1389
Tifton, GA 31793
Phone: 229-386-3340

Illinois
College of Veterinary Medicine
1231 VMBSB
Laboratories of Veterinary Diagnostic Medicine
2001 South Lincoln
Urbana, IL 61802-6199
Phone: 217-333-1620

Animal Disease Laboratory
Illinois Department of Agriculture
Shattuc Road
Centralia, IL 62801-5858
Phone: 618-532-6701

Animal Disease Laboratory
Illinois Department of Agriculture
2100 South Lake Storey Road
PO Box 2100X
Galesburg, IL 61402-2100
Phone: 309-344-2451

Indiana
Animal Disease Diagnostic Laboratory
School of Veterinary Medicine
Purdue University
West Lafayette, IN 47907
Phone: 765-494-7448

Iowa
Veterinary Diagnostic Laboratory
College of Veterinary Medicine
Iowa State University
Ames, IA 50010
Phone: 515-294-1950

Kansas
College of Veterinary Medicine
Veterinary Diagnostic Laboratory
Kansas State University
Manhattan, KS 66506
Phone: 785-532-5650

Kentucky
Murray State University
Veterinary Diagnostic and Research Center
PO Box 2000 North Drive
Hopkinsville, KY 42240
Phone: 270-886-3959

University of Kentucky
1429 Newtown Pike
Lexington, KY 40511
Phone: 859-257-9000

Louisiana
Veterinary Medical Diagnostic Laboratory
PO Box 25070
Baton Rouge, LA 70894
Phone: 225-578-9777

Michigan
Animal Health Diagnostic Laboratory
Michigan State University
PO Box 30076
Lansing, MI 48909-7576
Phone: 517-353-1683

Minnesota
Veterinary Diagnostic Laboratory
University of Minnesota
1943 Carter Avenue
St Paul, MN 55101
Phone: 612-625-8787

Missouri
Veterinary Medical Diagnostic Laboratory
University of Missouri
PO Box 6023
Columbia, MO 65205
Phone: 573-882-6811

Montana
State of Montana Animal Health Division
Box 997
Bozeman, MT 59771
Phone: 406-994-4885

Nebraska
Lincoln Diagnostic Laboratory
University of Nebraska
Lincoln, NE 68583-0907
Phone: 402-472-1434

New York
New York State College of Veterinary Medicine
Veterinary Diagnostic Laboratory
PO Box 5786
Ithaca, NY 14852
Phone: 607-253-3900

North Carolina
Rollins Animal Disease Diagnostic Laboratory
North Carolina Department of Agriculture
PO Box 12223
Cameron Village Station
Raleigh, NC 27605
Phone: 919-733-3986

North Dakota
North Dakota State University
Veterinary Diagnostic Laboratory
Van Es Hall
PO Box 5406
Fargo, ND 58105
Phone: 701-231-8307

Ohio
Division of Animal Health
Ohio Department of Agriculture
8995 East Main Street
Reynoldsburg, OH 43068-3399
Phone: 614-728-6220

Oklahoma
Animal Disease Diagnostic Laboratory
College of Veterinary Medicine
Oklahoma State University
PO Box 7001
Stillwater, OK 74074-7001
Phone: 405-744-6623

Pennsylvania
Department of Agriculture
State Veterinary Lab
2305 North Cameron Street
Harrisburg, PA 17110-9449
Phone: 717-787-8808

South Dakota
Animal Disease Diagnostic Laboratory
South Dakota State University
Veterinary Science Building
PO Box 2175
Brookings, SD 57007-1396
Phone: 605-688-5172

Texas
Veterinary Medical Diagnostic Laboratory
Texas A&M University
Box 3200
Amarillo, TX 79106
Phone: 806-353-7478

Texas Veterinary Medical Diagnostic Laboratory
Drawer 3040
College Station, TX 77841
Phone: 979-845-3414

Washington
Animal Disease Diagnostic Laboratory
Washington State University
PO Box 2037
Pullman, WA 99165-7034
Phone: 509-335-9696

Wisconsin
Wisconsin Veterinary Diagnostic Laboratory
Wisconsin Department of Agriculture
6101 Mineral Point Road
Madison, WI 53705
Phone: 608-262-5432

Wyoming
Wyoming State Veterinary Laboratory
1174 Snowy Range Road
Laramie, WY 82070
Phone: 307-742-6638

Canada
Guelph Ontario Ministry of Agriculture and Food
Veterinary Laboratory Services
Guelph Laboratories
Box 3612
Guelph, Ontario N1H 6R8
Canada
Phone: 519-823-8800, EXT 4501

Animal Health Monitoring Laboratory
1767 Angus Campbell Road
Abbottsford, British Columbia V3G 2M3
Canada
Phone: 604-556-3134

Another listing of animal disease laboratories arranged by state and city is available from the following source. Information provided includes the name of the laboratory, director,

address, phone, affiliation, who may submit specimens, major species accepted for examination, and the services offered. This source may be used to locate referral laboratory testing nearest to the reader. Send correspondence to:

National Veterinary Services Laboratories
Biometrics and Data Systems
PO Box 844
Ames, IA 50010

LABORATORY DESCRIPTIONS

Note that the full addresses listed here are not repeated under the descriptions of individual tests discussed later.

Animal Health Diagnostic Laboratory (AHDL)
Michigan State University
PO Box 30076
Lansing, MI 48909-7576
Phone: 517-353-1683

Service Role - State-supported diagnostic laboratory mainly serving Michigan veterinarians and animal owners.

Service Range - Pathology, clinical pathology, endocrinology, field investigation, immunology, microbiology, nutrition, parasitology, and toxicology.

Antech Diagnostics
17672-A Cowan Avenue, Suite 200
Irvine, CA 92714
Phone: 800-745-4725

Service Role - Large national commercial laboratory system with many regional laboratories.

Service Range-Wide range of testing.

Clinical Immunology Laboratory
University of Pennsylvania
School of Veterinary Medicine
Room 2016
3850 Spruce Street
Philadelphia, PA 19104
Phone: 215-898-6882

Service Role - University.

Service Range - Immunology (rheumatoid factor [RF], Coombs', immunoglobulins, immunofluorescent biopsy), endocrinology, feline infectious peritonitis (FIP) titer, *Aspergillus* antigen, and enzyme-linked immunosorbent assay (ELISA) for atopic disease in both the dog and cat.

Comparative Hematology Section
Diagnostic Laboratory
College of Veterinary Medicine
Cornell University
Upper Tower Road
Ithaca, NY 14853-5786
Phone: 607-253-3333
Fax: 607-253-3943

Service Range (Hemostasis) - Individual factor analysis, coagulation screening tests, coagulation inhibitor assays, von Willebrand factor (vWF), platelet aggregation, blood typing.

Diagnostic Laboratory
College of Veterinary Medicine and
Biomedical Sciences
Colorado State University
Fort Collins, CO 80523
Phone: 970-491-1281

Service Role - State diagnostic laboratory system.

Service Range - Clinical pathology, pathology, endocrinology, microbiology, toxicology, parasitology.

Diagnostic Laboratory
New York State College of Veterinary
Medicine Cornell University
PO Box 5786
Ithaca, NY 14852
Phone: 607-253-3900

Hansen Veterinary Immunology
450 Porter Road
Suite C
Dixon, CA 95620
Phone: 707-678-9680
Fax: 707-678-1582

Service Role - Commercial.

Service Range - Immunologic testing such as antinuclear antibody (ANA), immunofluorescence of tissue, titers for canine distemper, FIP, feline immunodeficiency virus (FIV), heartworm, and feline leukemia virus (FeLV).

IDEXX Veterinary Services
1 IDEXX Drive
Westbrook, Maine 04092
Phone: 888-433-9987

Service Role - Large national commercial laboratory system with many regional laboratories.

Service Range - Wide range of testing.

Infectious Disease Laboratory
University of Georgia
Department of Small Animal Medicine
College of Veterinary Medicine
Athens, GA 30602-7386
Phone: 706-542-5812
Fax: 706-542-6460

Service Role - Veterinary school–affiliated laboratory.

Service Range - Serologic testing for tick-borne diseases (*Ehrlichia canis*, Rocky Mountain spotted fever [RMSF], Lyme disease), canine and feline toxoplasmosis, FIV, and *Cryptococcus*.

Louisiana Veterinary Medical Diagnostic Laboratory
PO Box 25070
Baton Rouge, LA 70894
Phone: 225-578-9777
Fax: 225-578-9784

Texas Veterinary Medical Diagnostic Laboratory
Texas A&M University
College of Veterinary Medicine
Drawer 3040
College Station, TX 77841-3040
Phone: 979-845-3414
Fax: 979-845-1794

Service Role - State diagnostic laboratory system.

Service Range - Clinical and anatomic pathology, bacteriology, virology, and toxicology.

Tick-Borne Diseases Laboratory
North Carolina State University
College of Veterinary Medicine
Room C-321
4700 Hillsborough Street
Raleigh, NC 27606
Phone: 919-513-6357

Service Role - Veterinary school–affiliated laboratory.

Service Range - Indirect fluorescent antibody (IFA) end point titering for tick-borne diseases (*Ehrlichia canis* and *E. risticii,* RMSF, Lyme disease) and *Bartonella henselae* and *B. vinsonii*.

ENDOCRINE TESTS

Endocrine Diagnostic Section
Animal Health Diagnostic Laboratory
Michigan State University
B-619 West Fee Hall
East Lansing, MI 48824
Phone: 517-353-0621

Service Role - Endocrinology.

General Preparation of Samples. *Do not send whole or clotted blood. Label tubes with time of collection in timed procedures.* Thyroid hormones (except free T_4 [fT_4] by dialysis) are stable and need no refrigeration. Other hormones should be sent on ice packs. **For Serum:** Let blood clot in a plain glass tube at room temperature, centrifuge as soon as possible, remove serum, and refrigerate immediately. **For Plasma:** Collect in an EDTA tube, centrifuge within 15 minutes, remove the plasma, and refrigerate immediately. For shipment on ice (frozen gel packs) in an insulated container, wrap samples in soft paper toweling to protect from breakage, place samples in the middle of two to three frozen gel packs, and fill the container with crumpled paper or other material for insulation. Samples to be sent on ice should be frozen if not shipped on the day of collection.

Serum Thyroid Profile Sample. 2.0 ml of serum for canine thyroid profile and 1.5 ml for other species. Add 0.5 ml of serum for reverse T_3.

Therapeutic Monitoring. T_3 at 3 hours after Cytobin or T_4 at 3 to 8 hours after Soloxine. Indicate the therapy, dose, animal's weight, and time post-pill. Wait 1 month after discontinuing therapy before reevaluating thyroid function.

Cortisol Sample. 0.5 ml of plasma or serum separated within 30 minutes per sample on ice. ACTH response test. Collect a pretest resting sample, inject 0.25 mg cosyntropin total

dose intramuscularly (IM) and collect 1-hour post-ACTH sample. For low-dose dexamethasone suppression, collect a pretest sample, inject 0.01 mg of dexamethasone/kg IV, and obtain 4- and 8-hour post-dexamethasone samples (see Chapter 8).

Insulin Sample. 1 ml of serum shipped on frozen gel packs. For suspected insulinoma, collect a fasting sample for an insulin:glucose ratio.

Aldosterone Sample. 0.5 ml of EDTA plasma shipped on frozen gel packs. Collect a sample before and 1 hour after 0.25 mg cosyntropin IM.

Gastrin. 0.5 ml of serum shipped on ice. Include a resting sample after a 24-hour fast, then 15, 30, 45, and 60 minutes after feeding one half can P/D and one half can beef broth or a whole can if the dog weighs more than 40 pounds. Time zero is the start of feeding. Remove any remaining food by 15 minutes.

Parathormone and 25-Hydroxyvitamin D Sample. Collect fasting blood sample, lipemia must be avoided. Let blood clot at room temperature and remove at least 0.5 ml of serum within 1 hour. Ship immediately on 2 to 3 frozen gel packs by overnight express delivery. Mark form "SPECIAL HANDLING" in bold letters. Freeze serum if it cannot be shipped immediately.

Testosterone Sample. 1.0 ml of serum shipped on ice. For GnRH (Cystorelin) response, obtain pretest sample, inject 0.1 µg GnRH/lb intravenously (IV) and obtain 1- and 2-hour post samples.

Insulin-Like Growth Factor (Somatomedin C) Sample. 0.5 ml serum.

Plasma Renin Activity Sample. Obtain 1.5 ml EDTA blood in a chilled tube. Immediately cool on ice. Keep cold while centrifuging. Remove plasma, freeze, and ship on dry ice by overnight express delivery. Must arrive frozen. Mark form "SPECIAL HANDLING."

Estradiol (Estrogen)
Diagnostic Laboratory
Cornell University

Sample: 1 ml of serum.

Rothgerber Endocrinology Laboratory
Colorado State University
Fort Collins, CO 80523

Service Role. University.

Service Range. Wide variety of hormones, including estradiol.

Sample: Contact laboratory for information.

IMMUNOLOGIC TESTS

Antimegakaryocyte Antibody

School of Veterinary Medicine
University of California, Davis
Immunology Laboratory, Veterinary
Teaching Hospital
Davis, CA 95616
Phone: 530-752-7373
Fax: 530-754-9007

Sample: Bone marrow smears. Call laboratory.

Blood Typing

Midwest Animal Blood Services, Inc
PO Box 626
4983 Bird Drive
Stockbridge, MI 49285
Phone: 517-851-8244
Fax: 517-851-7762

Canine red blood cell (RBC) typing for identifying blood donors.

Sample: 3 ml EDTA blood and 0.5 ml serum. Ship cool, not frozen, by 24-hour courier. Full dog erythrocyte antigen (DEA) screen for 1.1, 1.2, 2, 3, 4, 5, and 7 plus screen sera for antibody to non-DEA type in each dog.

Feline RBC typing by tube agglutination for identifying blood donors.

NOTE: In-clinic kits (i.e., "cards") are available for typing dogs and cats from RapidVet, DMS Laboratories Inc, 2 Darts Mill Road, Flemington, NJ 08822, Phone: 800-567-4367, Fax: 908-782-0832.

Antech Diagnostics

Canine RBC typing:

Sample: Blood in EDTA. Results available in 4 to 7 days.

Feline RBC typing:

Sample: EDTA blood. Results available in 1 to 2 days.

Tissue Typing and Pedigree Substantiation

MSU Tissue Typing Laboratory
B 228 Life Science Building
Michigan State University
East Lansing, MI 48824-1317
Phone: 517-355-4616
Fax: 517-353-5436

Molecular pedigree substantiation:

Sample: 5 ml EDTA or ACD whole blood shipped by next-day courier.

Molecular evaluation for transplant; high-resolution testing of two MHC loci:

Sample: 5 ml EDTA or ACD whole blood shipped by next-day courier.

Immunoglobulin Quantitation

Clinical Immunology Laboratory
Veterinary Animal Disease Diagnostic Laboratory
1243 Veterinary Pathobiology Building
Purdue University
West Lafayette, IN 47907
Phone: 765-494-9676

Sample: 1 ml of serum.

Immunofluorescence of Tissue

Clinical Immunology Laboratory
University of Pennsylvania

Sample: Tissue in Michel's fixative.

Diagnostic Laboratory
New York State College of Veterinary Medicine
Cornell University
PO Box 5786
Ithaca, NY 14852
Phone: 607-253-3900
Fax: 607-253-3943

Sample: Tissue in Michel's fixative.

Rheumatoid Factor

Clinical Immunology Laboratory
School of Veterinary Medicine
University of Pennsylvania

Sample: Serum from 10 ml clot tube.

Myasthenia Gravis (Acetylcholine Receptor Antibody)

Comparative Neuromuscular Laboratory
Basic Science Building
Room 1057
University of California, San Diego
La Jolla, CA 92093-0612
Phone: 619-534-1537

Sample: 1 ml of serum with cold pack. Contact laboratory for submissions for other neuromuscular testing.

SEROLOGIC TESTS

Babesiosis

Tick-Borne Diseases Laboratory
North Carolina State University

Sample: 1 ml serum for IFA end point (cold packed, not frozen).

Texas Veterinary Medical Diagnostic Laboratory
Texas A&M University

Sample: 0.5 ml of serum.

Protatek Reference Laboratory
574 East Alamo Street, Suite 90
Chandler, AZ 85225
Phone: 480-545-8499
Fax: 480-545-8409

Sample: 1 ml of serum.

Bartonella henselae and *B. vinsonii*

Tick-Borne Disease Laboratory
North Carolina State University

Sample: 1 ml serum for IFA end point (cold packed, not frozen).

Borreliosis (Lyme Disease)

Infectious Diseases Laboratory
University of Georgia
Phone: 706-542-5812

Sample: 0.5 ml of serum for ELISA.

Colorado Veterinary Diagnostic Laboratory
Colorado State University

Sample: 0.5 ml of serum for ELISA.

Animal Health Diagnostic Laboratory
Michigan State University

Sample: 1 ml of serum.

Tick-Borne Diseases Laboratory
North Carolina State University

Sample: 1 ml of serum for IFA end point (cold packed, not frozen).

Canine Brucellosis

Diagnostic Laboratory
Cornell University

Sample: 2 to 3 ml of serum.

Colorado Veterinary Diagnostic Laboratory
Colorado State University

Sample: 0.5 ml of serum.

Canine Distemper Titer

Diagnostic Laboratory
Cornell University

Sample: Minimum of 0.1 ml of frozen cerebrospinal fluid (CSF) (on dry ice).

Colorado Veterinary Diagnostic Laboratory
Colorado State University

Sample: 0.5 ml of serum for serum neutralization.

Animal Health Diagnostic Laboratory
Michigan State University
Virology Section - Dr. Maes

Sample: 2 ml of serum.

Ehrlichia canis Titer

Laboratories of Veterinary Diagnostic Medicine
Illinois College of Veterinary Medicine

Sample: 1 ml of serum (cold packed, not frozen).

Tick-Borne Diseases Laboratory
North Carolina State University

Sample: 1 ml of serum for IFA end point (cold packed, not frozen).

Infectious Diseases Laboratory
University of Georgia
Phone: 706-542-5812

Sample: 0.5 ml of serum.

Texas Veterinary Medical Diagnostic Laboratory
Texas A&M University

Sample: 1 ml of serum.

Ehrlichia platys Titer

Louisiana Veterinary Medical Diagnostic Laboratory
Baton Rouge, LA

Sample: 1 ml serum. Contact laboratory for samples for serologic or other testing.

Ehrlichia risticii and *Ehrlichia equi* Titers

Tick-Borne Diseases Laboratory
North Carolina State University

Sample: 1 ml of serum for IFA end point (cold packed, not frozen).

Fungal Titer (Coccidioidomycosis, Cryptococcosis, Blastomycosis)

Comment: Various state health department laboratories may evaluate animal samples if human exposure is involved.

Antech Diagnostics
Test: Coccidioidomycosis.

Sample: 3 to 4 ml of serum.

Coccidioidomycosis Serology Laboratory
School of Medicine
University of California
PO Box 1440
Davis, CA 95617
Phone: 530-752-1757

Sample: 2 to 3 ml of serum.

Texas Veterinary Medical Diagnostic Laboratory
Texas A&M University

Test: Blastomycosis, coccidioidomycosis, or aspergillosis.

Sample: 0.5 ml of serum for fungal titer.

Test: *Cryptococcus* antigen.

Sample: 1.0 ml of serum.

Infectious Disease Laboratory
University of Georgia
Phone: 706-542-5812

Test: Cryptococcus.

Sample: 1 ml of serum, CSF, or aqueous humor.

Diagnostic Laboratory
Cornell University

Sample: 1 ml of serum.

Leptospirosis Titer

Animal Health Diagnostic Laboratory
Michigan State University

Sample: 10 ml of serum (acute and convalescent titers).

Metabolic Screening

Veterinary Hospital of University of Pennsylvania
3900 Delancey
Room 4027
Philadelphia, PA 19104-6010
Phone: 215-898-8076

Sample: Call the laboratory.

Neosporosis Titer

College of Veterinary Medicine
Auburn University
Room 122, Green Hall
Auburn, AL 36849-5519

Sample: 0.25 ml serum.

Rocky Mountain Spotted Fever Titer

Infectious Disease Laboratory
University of Georgia

Sample: 0.5 ml of nonhemolyzed canine serum on a frozen pack.

Tick-Borne Diseases Laboratory
North Carolina State University

Sample: 1 ml of serum for IFA end point (cold packed, not frozen).

Toxoplasmosis Titer

Animal Health Diagnostic Laboratory
Michigan State University

Sample: Minimum of 2 ml of chilled or frozen serum.

Infectious Disease Laboratory
University of Georgia
Phone: 706-542-5812

Sample: 1 ml of serum for both IgG and IgM.

Colorado Veterinary Diagnostic Laboratory
Colorado State University

Sample: 1 ml of serum for both IgG and IgM; 0.3 ml of aqueous humor or CSF in EDTA; 1 ml of serum, 0.3 ml of aqueous humor or CSF in EDTA for *Toxoplasma* antigen.

Trypanosoma cruzi Titer

Texas Veterinary Medical Diagnostic Laboratory
Texas A&M University

Sample: 1 ml.

Tularemia Titer

New Mexico Department of Agriculture
Veterinary Diagnostic Services
PO Box 4700
700 Camino de Salud, NE
Albuquerque, NM 87196-4700
Phone: 505-841-2576

Service Range: Full-service diagnostic laboratory.

Sample: 1 ml for tube agglutination.

THERAPEUTIC DRUG MONITORING

Clinical Pharmacology Laboratory
College of Veterinary Medicine
Texas A&M University

College Station, TX 77843
Phone: 979-845-9184

Sample: Contact laboratory for amount of serum and time relative to drug administration (see Chapter 18). Currently offers assays for gentamicin, amikacin, phenobarbital, phenytoin, potassium bromide, benzodiazepines, and digoxin.

TOXICOLOGIC TESTS

Animal Health Diagnostic Laboratory
Michigan State University
Toxicology Laboratory

Anions (Water) (Bromide, Nitrite, Nitrate, Sulfate)

Sample: 100 ml of water in bottles provided by an extension agent or public health official.

Anticoagulant Screen (Brodifacoum, Bromadiolone, Chlorphacinone, Coumachlor, Coumafuryl, Dicumarol, Diphacinone, Pindone, Warfarin)

Sample: 50 g of liver, stomach contents, bait; 5 ml of whole blood or serum.

Convulsant Screen (Bromethalin, Penitrem A, Roquefortine, Strychnine)

Sample: 50 g liver, stomach contents, bait (contact lab).

Cyanide

Sample: 100 g of forage; 20 g of muscle; 5 ml of blood.

Handling: Samples must be quick-frozen and received frozen.

Gas Chromatography/Mass Spectrometry (GC/MS) Consultative Workup (Drugs, Pesticides, Industrial Chemicals)

Sample: 10 ml of whole blood; 50 g of brain; 100 g each of stomach contents, liver, and kidney; 10 g of body fat; 400 g of suspected material.

Ethylene Glycol

Sample: 1 ml of serum; 50 g of liver; 5 ml of urine.

Insecticides (Chlorinated Pesticides)

Sample: 50 g of brain; 100 g each of stomach or rumen contents, liver, and kidney; 10 g of body fat.

Lead (Blood)

Sample: 2 ml of whole blood.

Mineral Analysis (ICP-AES)

Sample: Minerals in tissue or bile for Al, Sb, As, Ba, B, Ca, Cd, Cr, Co, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, P, Pb, Se, Tl, and Zn. Submit 100 g each of stomach contents, liver, kidney, and suspect material; 2 to 10 ml bile, 2 ml serum, 10 ml urine, 1 L of water in special bottles provided by an extension agent or public health official.

Mycotoxin Screen (Aflatoxins, Zearalenone, Vomitoxin)

Sample: 500 g of feed, kept dry or frozen.

Polybrominated Biphenyls (PBB), Polychlorinated Biphenyls (PCB)

Sample: 50 g of brain.

Strychnine

Sample: 50 g of liver, stomach contents, bait.

Toxin Screen (GC/MS)

Sample: Contact lab.

OTHER TESTS

Calculus Analysis

Minnesota Urolith Center
Department of Small Animal Clinical Sciences
College of Veterinary Medicine
University of Minnesota
St Paul, MN 55108
Phone: 612-625-4221
Fax: 612-624-0751

Sample: Calculi(us) shipped dry in unbreakable container. Contact center for preservation of urethral plugs.

Urinary Stone Analysis Laboratory
Department of Medicine
School of Veterinary Medicine
University of California, Davis
Davis, CA 95616

Sample: Calculi(us) shipped dry in sealed plastic bag within a mailing tube.

Hemostatic Testing

Comparative Hematology Section
Diagnostic Laboratory
College of Veterinary Medicine, Cornell University

Sample: Contact laboratory for complete instructions before submission. Submit 2 to 3 ml of citrated plasma for coagulation assays including (1) coagulation screening tests (activated partial thromboplastin time [APTT], prothrombin time [PT], thrombin clotting time [TCT]), (2) specific coagulation factor assays, and (3) coagulation inhibitor assays (antithrombin III, lupus anticoagulant assays). Submit 1 to 2 ml citrated plasma for vWF assays (vWF antigen, vWF multimer assay, ristocetin cofactor activity). Submit 2 to 3 ml citrate plasma for fibrinolysis testing (fibrinogen degradation product titer, plasminogen, and soluble fibrin monomer). Submit 1 to 2 ml EDTA whole blood for blood typing (*canine:* DEA 1.1, 1.2, 3, 4, 5, and 7; *feline:* Type A, B, AB). Plasma with hemolysis or clots is unacceptable. Special shipping with dry ice or frozen cold packs is necessary.

Trypsin-like Immunoreactivity

Gastrointestinal Function Test Laboratory
Texas A&M University
College of Veterinary Medicine
College Station, TX 77843-4474
Phone: 979-862-2861
Fax: 979-862-2864

Sample: 1 ml of serum after an overnight fast.

Vitamin E, Selenium, and Vitamin A

Clinical Nutrition Section
Animal Health Diagnostic Laboratory
Michigan State University

Sample: 0.5 ml of serum per vitamin, 1 ml serum for selenium. Contact laboratory for other tests.

Karyotyping of Hermaphrodites

Veterinary Cytogenetic Laboratory
Minnesota Veterinary Diagnostic Laboratory
College of Veterinary Medicine
1333 Gortner Avenue
St Paul, MN 55108
Phone: 612-625-8787

Sample: 10 ml of sterile *heparinized* blood (10 IU heparin/10 ml blood). Keep sample at room temperature and avoid temperature extremes in transit. Send by U.S. Express Mail to ensure delivery within 24 to 48 hours after collection. Contact lab before submission.

Taurine Quantitation

Amino Acid Analysis Laboratory
Department of Molecular Biosciences
School of Veterinary Medicine
1091 Haring Hall
University of California, Davis
Davis, CA 95616-8741
Phone: 530-752-0168

Sample: 1 ml of heparinized plasma or whole blood.

Carnitine Quantitation

Comparative Neuromuscular Laboratory
Basic Science Building, Room 2095
University of California, San Diego
La Jolla, CA 92093-0612
Phone: 858-534-1537

Sample: 3 ml of heparinized plasma and 10 ml of urine.

Appendix II

Harold Tvedten

Reference Values

These reference values are used by the Veterinary Clinical Center at Michigan State University. Literature values are referenced. *One preferably should use reference values specific for the laboratory or instrumentation used for deriving data for one's patients*. See appropriate chapters for details about tests, including full names for those abbreviated here. Canine values were derived from 120 apparently normal dogs and 40 apparently normal cats and using a Technicon H-1 analyzer.

Hematology Reference Values Technicon H-1 Hematology Analyzer

	TEST	UNITS	CANINE	FELINE
COMPLETE BLOOD COUNT	WBC	$\times 10^3/\mu l$	6.02–16.02	4.87–20.10
	RBC	$\times 10^6/\mu l$	6.15–8.70	6.12–11.86
	Hemoglobin	g/dl	14.1–20.0	9.0–15.6
	Hematocrit	%	43.3–59.3	29.3–49.8
	MCV	fl	63.0–77.1	41.9–54.8
	MCH	pg	21.1–24.8	12.5–17.6
	MCHC	g/dl	29.9–35.6	28.1–32.0
	Platelets	$\times 10^3/\mu l$	164–510	26–470*
	MPV†	fl	3.9–6.1	4.1–8.3
	RDW†	%	11.9–14.9	14.2–17.6
	HDW†	g/dl	1.49–2.17	1.71–2.41

*See other platelet range of 230-680 $\times 10^3/\mu l$ listed later, which is a more accurate range. This reference range extends down into the thrombocytopenic range, which illustrates the problems of automated platelet counts on routinely collected feline blood samples.
†Using the multispecies software 2.0.

Absolute Differential Leukocyte Counts

	TEST	UNITS	CANINE	FELINE
DIFFERENTIAL	Neutrophils	$\times 10^3/\mu l$	3.23–10.85	2.5–12.5
	Lymphocytes	$\times 10^3/\mu l$	0.53–3.44	1.5–7.0
	Monocytes	$\times 10^3/\mu l$	0.0–0.43	0.0–0.85
	Eosinophils	$\times 10^3/\mu l$	0.0–1.82	0.0–1.50
	Basophils	$\times 10^3/\mu l$	0.01–0.54	0
	LUC*	$\times 10^3/\mu l$	0.26–2.09	N/A

Canine reference ranges are based on automated leukocyte differential counts of a Technicon H-1 analyzer on blood from 120 apparently normal dogs.
Note: Do not use automated differential leukocyte count reference ranges for interpreting manual differential leukocyte counts.
*LUC, large unstained cell in automated leukocyte differential (Technicon H-1).
Feline manual total leukocyte differential cell counts are from Jain NC: Schalm's Veterinary Hematology. 4th ed. Philadelphia, Lea and Febiger, 1986.

Coagulation Reference Values

	TEST	UNITS	CANINE	FELINE
COAGULATION	Platelets*	$\times 10^3/\mu l$	166–575	230–680
	PT*	seconds	5.1–7.9	8.4–10.8
	APTT*	seconds	8.6–12.9	13.7–30.2
	Fibrinogen	mg/dl	100–245	110–370
	FDP	µg/ml	<10	<10

*Coagulation tests are performed on the Fibrometer System.
Feline values are from Killingsworth C: Screening coagulation tests in the cat. Vet Clin Pathol 1985;14:19-23.

Chemistry Reference Values

TEST	UNITS	CANINE	FELINE
Arterial blood gas			
pH		7.36–7.44	7.36–7.44
P_{CO_2}	mm Hg	36–44	28–32
P_{O_2}	mm Hg	90–100	90–100
T_{CO_2}	mEq/L	25–27	21–23
HCO_3	mEq/L	24–26	20–22
Venous blood gas			
pH		7.34–7.46	7.33–7.41
P_{CO_2}	mm Hg	32–49	34–38
P_{O_2}	mm Hg	24–48	35–45
T_{CO_2}	mEq/L	21–31	27–31
HCO_3	mEq/L	20–29	22–24
A:G ratio (calculated)		0.89–2.68	0.80–1.68
Albumin	g/dl	3.2–4.7	3.0–4.6
ALP (alkaline phosphatase)	IU/L	0–90	4–81
ALT (SGPT)	IU/L	10–94	23–109
Ammonia (resting)	µg/dl	25–92	30–100
Amylase	IU/L	371–1503	531–1660
AST (SGOT)	IU/L	10–62	14–41
Bile acid (fasting)	µmol/L	0.0–15.3	0.0–7.6
Bile acid (2 hour)	µmol/L	0.0–20.3	0.0–10.9
Bilirubin—total	mg/dl	0.1–0.6	0.1–0.7
BSP		0–5%	0–5%
BUN	mg/dl	7–32	18–41
Calcium	mg/dl	9.0–11.9	8.4–11.5
Cholesterol	mg/dl	116–317	64–229
CK	IU/L	51–529	91–326
Creatinine	mg/dl	0.5–1.4	0.7–2.2
Electrolyte profile			
Sodium (Na)	mEq/L	146–156	153–162
Potassium (K)	mEq/L	3.9–5.5	3.6–5.8
Chloride (Cl)	mEq/L	113–123	119–132
T_{CO_2}	mEq/L	16.9–26.9	12.5–24.5
Anion gap		9–22	10–27
GGT	IU/L	1–6	1–3
Globulin	g/dl	1.5–3.5	2.1–4.0
Glucose	mg/dl	53–117	57–131
LDH	IU/L	42–130	63–193
Lipase	U/L	90–527	
Lipase	Sigma-Tietz units	0.1–1.3	0.1–0.4
Magnesium	mg/dl	1.36–2.09	1.38–2.36
Osmolality—serum			
Calculated	mOsm/Kg	302–325	319–371
Determined	mOsm/Kg	293–321	290–320
Osmolality—urine	mOsm	200–2000	200–2000
Phosphorus	mg/dl	1.9–7.9	2.9–8.3
SDH	IU/L	5.4–33.3	0.4–10
Total protein	g/dl	5.3–7.6	5.5–7.7
Triglyceride	mg/dl	10–500	10–500
Uric acid	mg/dl	0–1	0–1
Serum iron (Abbott)	µg/dl	61–255	34–122
Iron profile*			
Total iron	µg/dl	84–233*	68–215
UIBC	µg/dl	142–393	105–205
TIBC	µg/dl	284–572	†
Saturation	%	20–59	†

*Harvey JW, French TW, Meyer DJ: Chronic iron deficiency anemia in dogs. *JAAHA* 1982;18:946–960.
†Values not directly determined.

Selected Factors to Convert Commonly Used Units to the International System of Units

SUBSTANCE	COMMON UNIT	×	CONVERSION FACTOR	=	INTERNATIONAL UNIT
Albumin (proteins)	g/dl		10		g/L
Ammonia	µg/dl		0.587		µmol/L
Bicarbonate	mEq/L		1		mmol/L
Bile acids	µg/ml		2.45		µmol/L
Bilirubin	mg/dl		17.1		µmol/L
Calcium	mg/dl		0.25		mmol/L
Total CO_2	mEq/L		1		mmol/L
P_{CO_2}	mmHg		0.133		kPa*
Cholesterol	mg/dl		0.026		mmol/L
Chloride	mEq/L		1		mmol/L
Creatinine	mg/dl		88.4		µmol/L
Folate	ng/ml		2.27		nmol/L
Glucose	mg/dl		0.0555		mmol/L
Insulin	µIU/ml		0.0417		µg/L
Iron	µg/dl		0.179		µmol/L
Magnesium	mg/dl		0.411		mmol/L
P_{O_2}	mm Hg		0.133		kPa*
Phosphate	mg/dl		0.323		mmol/L
Potassium	mEq/L		1		mmol/L
Sodium	mEq/L		1		mmol/L
Urea nitrogen	mg/dl		0.357		mmol/L
Xylose	mg/dl		0.067		mmol/L
Enzymes	IU/L		0.017		µkat/L†
Amylase	Somogyi units/dl		1.85		IU/L
ALT (SGPT)	Karmen units/ml		0.48		IU/L
Lipase	Cherry-Crandall units/ml		278		IU/L
Blood cells	Cells/µl		1,000,000		Cells/L‡

*kPa, kilopascal.
†1 kat, 1 katal (i.e., 1 mol/sec), is for reporting enzyme activity, but most laboratories report serum enzyme activity in international units of IU/L or U/L (i.e., 1 µmol/min).
‡Reported as 10^9 cells/L.
Modified from Kaneko JJ: Clinical Biochemistry of Domestic Animals. 3rd ed. New York, Academic Press, 1980, pp 785-791; Lehmann HP, Henry JB: SI units. *In* Henry JB (ed): Clinical Diagnosis and Management by Laboratory Methods. 17th ed. Philadelphia, WB Saunders, 1984, pp 1428-1450.

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